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SOME VIRUSES AFFECTING GLADIOLUS[1]

G. H. Berkeley

In the fall of 1949, plants of the gladiolus variety Mrs. Mark's Memory were observed which showed brilliant chlorotic rings and patterns on older leaves, symptoms unlike any recorded disease affecting gladiolus (Fig. 1). Corms from affected plants of this variety and from several other varieties showing various degrees of leaf mottling and markings were collected and planted in the greenhouse in February, 1950. Further samples of these and other source material were collected again in the fall of 1950. An account of the viruses obtained and the diseases they caused in gladiolus comprises the present paper.

In 1928 Louise Dosdall (6) described a mosaic disease of gladiolus characterized by warty corms. Attempts to transmit the disease by juice transfer were negative. Smith and Brierley (9) also were unable to transmit a mosaic disease of gladiolus by juice transfers but they were able to transmit it with the vectors *Myzus persicae* and *M. circumflexes*. This virus was later identified as bean virus 2 (4). In the meantime, McWhorter et al. (8) proved by juice transfer that this virus infected gladiolus.

A mosaic disease of gladiolus, caused by cucumber mosaic virus, was reported from Tasmania in 1948 (10). In North America, strains of cucumber mosaic virus and tobacco ringspot virus have been isolated from gladiolus (1, 2, 3, 5), and in some instances transmitted back to it (3, 5) although the inoculated plants remained symptomless, except that those inoculated with one isolate of cucumber mosaic virus (5) produced yellowish flecks and gray lines in the leaves and break in the bloom.

MATERIALS AND METHODS.—The viruses were isolated from newly emerged 1–2 in. long shoots of naturally infected corms by grinding the shoot material with a mortar and pestle and squeezing the juice through several layers of cheesecloth.

The identity of the viruses thus obtained was based on symptomatology on a range of hosts, on thermal inactivation of the viruses, and on cross protection tests. The following host range was used: *Antirrhinum majus* L., *Nicotiana tabacum* L., var. Harrow Velvet, *N. glutinosa* L., *Datura stramonium* L., *Capsicum frutescens* L., var. California Wonder, *Lycopersicon esculentum* Mill., var. Grand Rapids, *Petunia hybrida* Vilm., *Phaseolus vulgaris* L., var. Bountiful, *Pisum sativum* L., var. Laxtons Progress, *Vigna sinensis* Savi., *Gomphrena globosa* L., and *Cucumis sativus* L., var. Windermoor.

One-year-old corms grown from seed of Corona, Carillon and King Lear varieties were inoculated with the viruses isolated from gladiolus. Each corm was cut into 2 equal seed-pieces, each of which was planted in a 5 in. pot. When the sprouts were 1–3 in. long, one of each pair of plants was inoculated by injection with a hypodermic needle at the base of the shoot in the vicinity of the growing points. The corresponding plant was left as a check. In some cases the young shoots of injected corms were also inoculated with the same inoculum by means of the carborundum juice transfer method. The inoculum was prepared by grinding symptom-bearing leaves of either tobacco or snapdragon and squeezing the juice through several layers of cheesecloth.

Because of the difficulty in obtaining virus-free gladioli, this technique has several distinct advantages. In the first place, natural infections of the original corm could be readily detected either by the development of symptoms on, or by transfers to suitable hosts from, the sister-plants used as checks. In many instances sister-check plants did show virus symptoms later. Isolations therefrom often made it possible to identify the virus naturally occurring in the original corms and to correlate the respective syndromes with the complex of viruses known to be present in the inoculated plants.

IDENTIFICATION OF VIRUSES ISOLATED FROM GLADIOLUS.—*Bean virus 2.*—This virus was isolated on 6 occasions from the varieties Kestrel, Pennant, and 3 unknown varieties, as well as from 1-year-old gladiolus seedlings. In addition, it was isolated along with a strain of tobacco ringspot virus from a single Kestrel plant and from a plant of an unknown variety. The symptoms on the source plants were chiefly of the mosaic type, though varying in degree. In Kestrel, the older leaves showed ivory to white spotting, while in Pennant the early outstanding symptoms were vertical lines and blocks.

Of the complete host range, only pea and bean were susceptible. On bean, the symptoms consisted of primary chlorotic spots, vein-banding, and mosaic of the yellow type. Leaf surfaces were rough, sometimes puckered, and non-necrotic. The symptoms on pea were also of the mosaic type.

This virus was inactivated when exposed for 10 minutes at a temperature of 59°C. Beans were used as test plants.

Cucumber mosaic virus.—This virus was obtained from the variety Spotlight, from 2 unknown varieties, and from a 1 year-old gladiolus seedling.

On tobacco, *Nicotiana glutinosa*, pepper, tomato, cucumber and petunia the symptoms were of the type associated with cucumber mosaic virus on these hosts, except that systemic necrosis was much more severe on *N. glutinosa*. On snapdragon and *Datura stramonium* the virus at times caused indistinct and indefinite primary chlorotic lesions. Bean and pea

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were insusceptible.

The thermal inactivation point of this virus when tested on tobacco was 71°C. for a 10-minute exposure.

Tobacco ringspot virus.—Three strains, designated A, B, and C were recovered from 5 gladiolus varieties, namely, Mrs. Mark's Memory, Picardy, Spotlight, Kestrel, an unknown variety, and from several gladiolus seedlings. Strain A used in these investigations was obtained from Mrs. Mark's Memory, strain B from an unknown variety, and strain C from the Kestrel variety.

The syndromes induced by strains A, B, and C on the respective hosts varied considerably in degree and effect but, in general, resembled those associated with tobacco ringspot virus on these hosts (11).

On tobacco, strain A induced minute whitish primary rings, minute spots, and sometimes small chlorotic rings followed by systemic chlorotic rings, spots and patterns with moderate necrosis in the margins of the chlorotic areas, whereas strain B produced large necrotic spots or rings, followed by systemic necrotic spots or rings with considerable distortion of leaves. In contrast, strain C generally produced no symptoms or at best only a few large indistinct chlorotic primary rings and equally few and indistinct secondary rings. That such symptomless plants were infected and were carriers of this strain was demonstrated by recovery of this strain on snapdragon, bean, or cucumber.

On *N. glutinosa*, strains A and C generally produced no symptoms although on occasion a few chlorotic or necrotic primary spots developed. There were no systemic symptoms. Strain B, on the other hand, caused primary necrotic spots and/or whitish or green etched rings followed by systemic chlorotic to whitish etched rings and patterns. It was established that *N. glutinosa* was often a symptomless carrier of strains A and C.

On snapdragon strains A and B produced primary tan necrotic spots, rings or patterns with ultimate necrosis of the rubbed leaves. Necrotic areas also developed on petioles and stems. Though systemic symptoms were sometimes lacking, inoculated plants often developed necrotic spotting and some necrosis of tip growth. However, inoculated plants were rarely killed. With strain C, however, necrosis was absent and symptoms were confined to etched rings and patterns on the rubbed leaves. Systemic symptoms were confined to etched rings and patterns without necrosis.

On bean, strains A and B produced necrosis of veins and fine necrotic spotting on young primary leaves followed by severe systemic spot necrosis, casting of true leaves, and killing of growing points. With strain C, however, necrosis was confined to mild spotting of first true leaves. Later formed leaves became mottled and rough and at times the tip leaves were mildly distorted. Leaves were not cast and growing points were unaffected.

On cucumber, all 3 strains caused primary chlorotic lesions on cotyledons followed by conspicuous chlorotic spotting of the first true leaves. In addition, however, strain B produced minute systemic necrotic rings and necrotic spotting of leaves, which became stunted with margins turned upward and inward.

On cowpea all 3 strains produced small reddish primary lesions on rubbed leaves, which later wilted and dropped off. In addition, strains A and B caused systemic necrosis and often death of plants, whereas strain C caused only a faint general mottle with mild waviness of leaf margins.

On *Gomphrena globosa* strains A and C produced primary chlorotic lesions, with or without necrotic centers. Systemic symptoms were either lacking or consisted of a few chlorotic spots and mild waviness of leaf margins. With strain B, the local lesions were larger and were necrotic as well as chlorotic, and systemic symptoms consisted of whitish necrotic spots and rings on the upper leaves, which became twisted and mildly distorted.

On pea, *Datura stramonium*, and petunia the symptoms caused by all 3 strains of the tobacco ringspot virus were of about the same degree and type. Pepper and tomato were not infected by any of the 3 strains.

From this comparison of syndromes it is apparent that strain B produced the most severe symptoms on all hosts, whereas strain C caused the mildest reaction and strain A was intermediate in its effect.

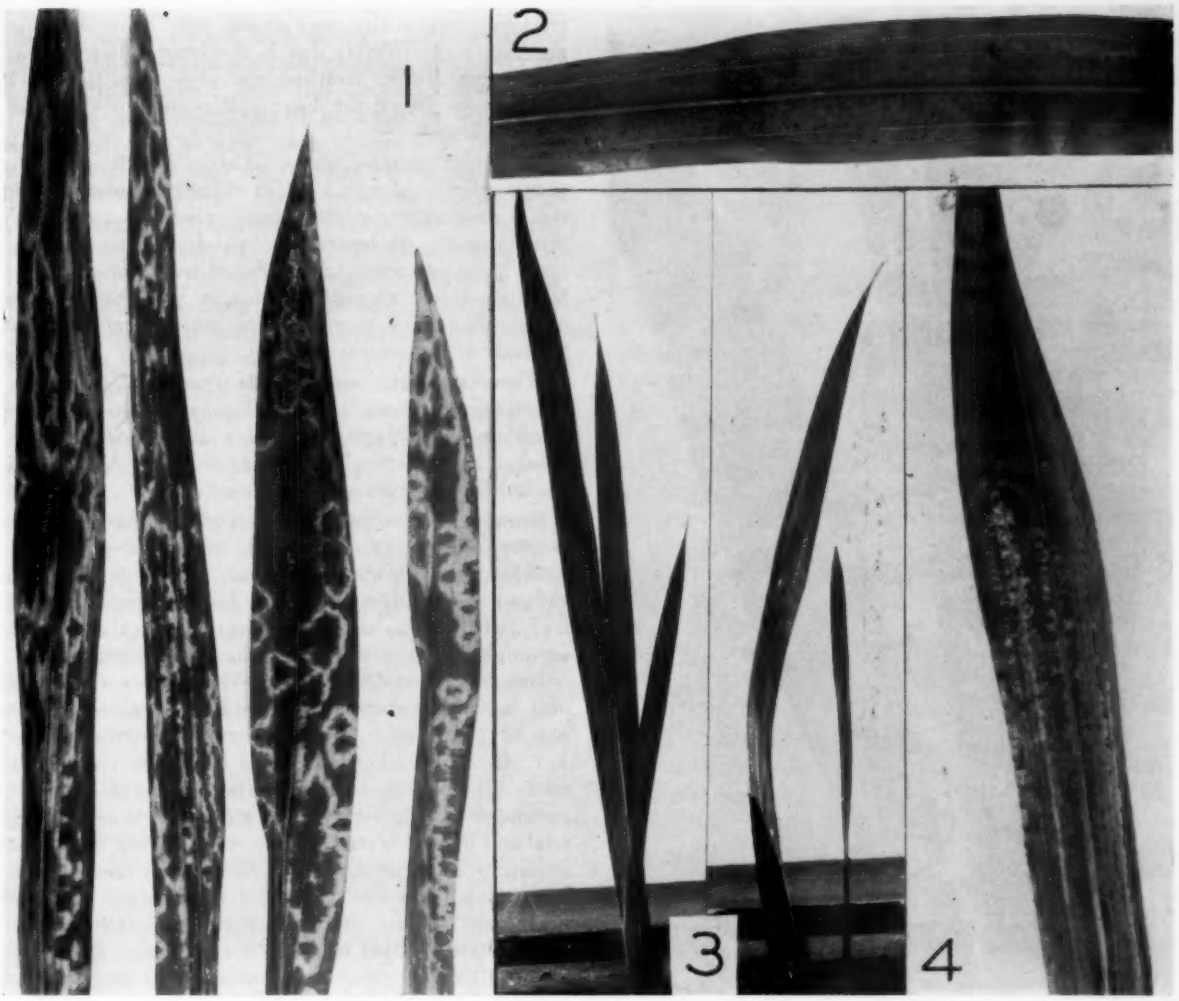
Strain A may be identified by the chlorotic symptoms on tobacco and *Gomphrena globosa*, and the lack of symptoms on *Nicotiana glutinosa*, while strain B may be identified by the necrotic symptoms on tobacco, *Gomphrena globosa* and cucumber, and the primary and systemic symptoms on *N. glutinosa*. Strain C may be identified by the almost complete lack of symptoms on tobacco, etching only on snapdragon, and the mild systemic symptoms on bean and cowpea.

On the basis of symptoms, strain A may be closely related to the green strain of the tobacco ringspot virus, whereas strain B more closely resembles the typical virus. Strain C, however, would appear to be a new strain of this virus.

Cross protection tests.—In 3 successive tests in triplicate, tobacco plants that had recovered from infection with a typical strain of tobacco ringspot virus showed no further symptoms when inoculated with any one of the 3 gladiolus strains. Similar recovered plants were not protected against either the cucumber mosaic virus isolated from gladiolus or tobacco mosaic virus. Since tobacco is often symptomless when infected with strain C, additional tests with this strain were carried out on bean in place of tobacco. Protection against tobacco ringspot virus on bean by strain C verified that strain C was a strain of tobacco ringspot virus.

Thermal inactivation tests.—The thermal inactivation point of strains A and B was found to be 61°C., whereas that of strain C was 57°C. The thermal inactivation point of the tobacco ringspot virus used in these tests was 66°C.

These inactivation temperatures are slightly lower than that reported for the typical virus, 68°C., or that for the tobacco ringspot strain isolated by Bridgmon and Walker (3) from gladiolus, 65–70°C.



FIGS. 1–4. Fig. 1. Symptoms of brilliant chlorotic rings and patterns on leaves of the gladiolus variety Mrs. Mark's Memory.—Fig. 2. Chlorotic mottling induced on gladiolus leaf by strain A of tobacco ringspot virus.—Fig. 3. Symptoms caused by strain B of tobacco ringspot virus on gladiolus; (left) sister-check plant, (right) inoculated plant.—Fig. 4. Symptoms caused by strain B on gladiolus showing whitish specking in some detail. Note rings and spots.

INOCULATION OF GLADIOLUS SEEDLINGS WITH THE VIRUSES ISOLATED FROM GLADIOLUS.—*Strains A, B, and C of tobacco ringspot virus.*—Ten out of 20 gladiolus seedlings inoculated with strain A responded to infection by producing definite chlorotic mottling on the first and sometimes on the second leaves. The mottling, though definite, was not severe and consisted of light-green to dark-green spots and blocks (Fig. 2). Later formed leaves were symptomless or nearly so.

Fifteen out of 20 gladiolus seedlings inoculated with strain B reacted to infection by producing well-defined whitish specking in the form of dots, rings, and mottled areas towards the base of the first and sometimes of the second leaves (Figs. 3 and 4). Later formed leaves did not show this specking. Inoculated plants were moderately stunted.

Strain C, on the other hand, produced no definite symptoms on inoculated gladiolus seedlings.

The respective check plant of each plant inoculated with either strain A, strain B, or strain C remained symptomless, and transfers from these check plants to tobacco, cucumber and bean were negative.

On the other hand, when transfers were made from the inoculated plants to tobacco, cucumber, and bean, the strain used for inoculation was readily recovered.

Thus it would appear that strain A caused a mild mosaic disease on gladiolus, while a more severe whitish specking disease resulted from infection with strain B. With strain C no symptoms resulted, but the virus was readily recovered from inoculated plants.

In addition to the inoculations with the gladiolus strains of the tobacco ringspot virus, 3 plants of the King Lear variety were inoculated with an isolate of this virus from field tobacco. Two of the 3 plants responded to infection by producing a chlorotic mottling similar to that caused by strain A. The 2 check plants remained symptomless, but no isolations were made therefrom.

FIG. 5. Symptoms on gladiolus caused by cucumber mosaic virus. Note pronounced mottling with streaks occurring on all leaves.

Complex of viruses.—In the course of these investigations many of the check plants showed mottling of foliage after their respective sister-plants had been inoculated. Most of these pairs of plants were discarded, but 5 plants inoculated with strain A produced the white specking syndrome associated previously with strain B. Transfers were made both from the inoculated plants and from their sister-checks to tobacco, cucumber and bean, and in some cases to snapdragon. Strain A was readily recovered from the 5 inoculated plants and from 1 of them the bean yellow mosaic virus also. From the 5 sister-check plants only the bean yellow mosaic virus was recovered. In these 5 cases, it would appear that the white specking symptom was due to infection by a mixture of tobacco ringspot strain A and the bean yellow mosaic virus. The primary specking symptom caused by this virus complex was similar to that caused by strain B alone. On the other hand, the later formed leaves showed mild mottling, which was absent with strain B. This mottling was probably due to the bean yellow mosaic virus, since similar mottling was present on the check plants from which the bean yellow mosaic virus had been recovered.

Cucumber mosaic virus.—Three of 10 gladiolus plants inoculated with a strain of the cucumber mosaic virus originally isolated from gladiolus, produced symptoms on all leaves that unfolded after inoculation. These symptoms also differed from those induced by the tobacco ringspot strains in that they were a more pronounced grayish streak mottle that persisted throughout the season (Fig. 5).

When transfers were made back to cucumber, *Nicotiana glutinosa*, bean, and tobacco, the resulting symptoms were typical of those associated with cucumber mosaic virus on these hosts. The sister-check plants were symptomless and no virus was recovered.

Bean yellow mosaic virus.—Only a few gladiolus seedlings were inoculated with this virus and indefinite results were obtained. Only 2 inoculated plants showed mild indistinct mottle on the first or second leaf, but the virus was not recovered from these plants although transfers were made on several occasions.

DISCUSSION.—Before 1951 the only disease of gladiolus in North America proved to be of virus origin was a mild mosaic caused by the bean yellow mosaic virus (8). In 1951 Berkeley (1) and Bridgmon (2) reported for the first time the isolation from gladiolus of cucumber mosaic and tobacco ringspot viruses in Canada and in the United States respectively. In 1952 Brierley (5) confirmed the occurrence of these viruses in gladiolus and was successful in infecting gladiolus with both viruses, although he reported that tobacco ringspot virus failed to produce symptoms. Bridgmon and Walker (3) also were unsuccessful in obtaining symptoms with this virus.

In the present investigations, only 2 of the 3 strains of tobacco ringspot virus induced symptoms on gladiolus seedlings, although all 3 strains were successfully transferred to gladiolus. These results would indicate that the presence or absence of symptoms depends on the strain of the virus used and provide a possible explanation for the negative results reported by Brierley (5) and Bridgmon and Walker (3). However, it is probable that expression of virus symptoms by gladiolus may also be affected by other factors, such as varietal reaction to infection and the method of inoculation.

It should be pointed out that strains of tobacco ringspot virus have been isolated from gladiolus showing varying degrees of mottling, as well as from the variety Mrs. Mark's Memory showing definite chlorotic rings and patterns, and from the Kestrel variety with severe whitish specking of the older leaves. Therefore, to date, there has been no definite correlation between the virus or viruses isolated and the syndromes on the gladioli from which they were isolated. For example, strain A was isolated from a gladiolus showing brilliant chlorotic rings and patterns, but the

symptoms caused by inoculation of gladiolus seedlings with this strain were a mild mosaic. Likewise strain C was isolated from a gladiolus showing whitish specking, yet inoculations with this strain produced no symptoms on gladiolus seedlings although the plants were infected. The most likely explanation for these variable results is that the original plants contained a mixture of viruses, only one of which was isolated, or the differential reaction might conceivably be related to use of seedlings rather than the gladiolus variety concerned. That gladioli are commonly infected with virus mixtures has been well established by Brierley (5) and Bridgmon and Walker (3) and verified in the present investigation.

There appears to be some correlation between the type of symptom produced by the tobacco ringspot strains on tobacco and other hosts and that produced on gladiolus. For instance, strain A caused mainly chlorotic symptoms on both tobacco and gladiolus, whereas strain B produced necrotic symptoms on tobacco and whitish specking or necrotic-like etching on gladiolus. Likewise, strain C produced no symptoms on gladiolus and mild symptoms only rarely on tobacco.

Like certain other hosts of tobacco ringspot virus, gladiolus reacts to infection by expressing symptoms on newly infected leaves, but leaves formed after the growing points become infected show little to no symptoms.

It has been commonly observed in the field that Kestrel and certain other varieties show whitish specking in varying degrees mainly on the older leaves, especially on small plants grown from cormels. The symptoms caused by strain B appear to be of this type. However, similar symptoms may arise from other causes. for example, when plants infected with bean yellow mosaic virus later became infected with strain A. Plants infected with this mixture of viruses, however, unlike those infected with strain B alone, showed mild mottling on the later formed leaves, probably due to the bean yellow mosaic virus.

It should be pointed out that bean yellow mosaic virus isolated from the several Ontario sources produced yellow mosaic symptoms, without necrosis on the variety of bean used. So far, the necrogenic strain reported by McWhorter and Boyle (7) in Oregon has not been encountered on gladiolus in Ontario. On the other hand, severe systemic necrosis, top necrosis, and death of bean plants were induced by 2 strains of tobacco ringspot virus.

SUMMARY

Bean yellow mosaic virus, strains A, B, and C of tobacco ringspot virus, and cucumber mosaic virus have been isolated from gladiolus. Their identity was established by their symptom expression on snapdragon, tobacco, *Nicotiana glutinosa*, jimson weed, bean, pepper, cucumber, pea, tomato, petunia, cowpea and globeamaranth, by thermal inactivation points, and, for the tobacco ringspot strains, by cross protection tests as well.

Strain A of tobacco ringspot virus produced on rubbed tobacco leaves minute whitish rings and systemic chlorotic rings and patterns. On snapdragon and bean it produced severe necrosis.

Strain B was identified by severe necrosis and distortion on tobacco and by necrosis on cucumber.

Strain C was almost symptomless on tobacco and caused only surface etching on snapdragon and mild symptoms on cowpea and bean.

The thermal inactivation points of these viruses from gladiolus were: bean virus 2, 59°C.; cucumber mosaic virus, 71°C.; and the tobacco ringspot strains, 57°C. for strain C and 61°C. for strains A and B.

One-year-old seedling gladiolus plants were inoculated with the viruses isolated from gladiolus. Strain A of tobacco ringspot virus, and bean yellow mosaic virus caused mild chlorotic mottling, whereas strain B of tobacco ringspot virus produced whitish specking. These symptoms occurred mainly on the leaves developing immediately after inoculation. Strain C of tobacco ringspot virus caused no symptoms on gladiolus but was readily recovered from inoculated plants.

Strain A in combination with bean yellow mosaic virus produced whitish specking symptoms similar to those caused by strain B alone.

The strain of cucumber mosaic virus tested caused prominent and persistent mottling with grayish streaks on all leaves as they unfolded.

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THE PHYSIOLOGY OF FUSARIUM WILT OF TOMATO[1]

R. P. Scheffer and J. C. Walker

Several theories have been offered to account for wilting of tomato (*Lycopersicon esculentum* Mill.) plants infected with *Fusarium oxysporum* f. *lycopersici* Snyder and Hansen. Among the suggested explanations are various processes of plugging of vessels, e.g., by mycelial growth of the pathogen, by tyloses from adjacent cells, and by gums produced either by the host or by the pathogen. Another explanation has been the production by the pathogen of toxins carried by the transpiration stream beyond the area of infection. This last theory has in recent years become rather widely accepted, but Ludwig (9) has recently found evidence indicating that plugging is a major factor in producing wilt.

The toxin theory has been expanded recently by Gäumann and co-workers, who have identified a peptide, lycomarasmin, as the agent repsonsible for wilting. A review of the lycomarasmin work has been published (2), but several points pertinent to the present work will be discussed here. Since the toxin has never been identified in diseased plants, the theory rests on the action of purified lycomarasmin on cuttings from tomato plants. Lycomarasmin was isolated from filtrates of 2- to 4-months-old cultures of the pathogen. Two strains of the latter obtained from the U. S. Department of Agriculture, were compared as to the amount of lycomarasmin produced. Strain R5-6, a very virulent strain *in vivo*, produced little lycomarasmin *in vitro*, while strain 257, almost non-pathogenic *in vivo*, gave a high yield of the polypeptide *in vitro* (5). Lycomarasmin has not been found in other forms of *Fusarium*, or in other species of fungi. No parallel was established between resistance and susceptibility of tomato varieties to *Fusarium* wilt and sensitivity to lycomarasmin injury. The varieties Bonny Best (very susceptible), Marglobe and Rutgers (moderately susceptible), and Pan America and Red Currant (*Lycopersicon pimpinellifolium* Dunal) (highly resistant) were all equally sensitive to the purified toxin (5).

Symptoms of lycomarasmin injury first appeared on the tips and margins of the leaves. Two to 4 hours after tomato cuttings were placed in a 10^{-3} molar solution of lycomarasmin, affected areas showed a dull, dark color, gradually shriveled, and became desiccated. Subsequently, injury proceeded inwardly from the leaf margin, until completely dried leaflets hung on petioles which were still fresh and turgid. The main stems of the cuttings remained normal in turgidity. Lycomarasmin did not injure vascular bundles, xylem parenchyma, phloem or cortical tissue of stem or petiole, but exerted its action on the cells of the leaf blade (3). It also had a striking effect on the transpiration and water absorption of tomato cuttings. Several phases in the toxic action were observed. Two to 3 hours after the cut ends of tomato shoots were placed in a solution of lycomarasmin, there occurred a shock phase with a repression of water absorption and transpiration. Concentration of lycomarasmin affected the intensity but not the occurrence of the shock phase. The second phase was characterized by excessively high transpiration which greatly exceeded absorption, indicating that cell contents were passing into the transpiration stream. Five to 8 hours after exposure to the toxin, the excessive transpiration phase was followed by a collapse of water economy in which transpiration and absorption dropped to low levels. When 10^{-2} and 10^{-3} M solutions of lycomarasmin were used, water loss continued to exceed the uptake until wilting occurred, usually within 5–10 hours after the second phase had ended. No wilting occurred when 10^{-4} or 10^{-5} M solutions were used, but transpiration continued to be above normal (3). Wilt was also induced by lycomarasmin in a saturated atmosphere where there was no transpiration, showing that the wilt was not directly related to water loss (3).

When a small amount of free iron was added to the lycomarasmin solution, the toxicity of the latter was increased approximately 10 times. Thus 150 mg. of pure lycomarasmin per kg. of green weight was required for a minimum toxic dose while only 10–15 mgs. of lycomarasmin was required when free iron was present (4). It was suggested that a lycomarasmin-iron complex was formed which was more toxic than pure lycomarasmin (5). Although the nutrition of the host had been found to influence significantly the development of *Fusarium* wilt of tomato (12), cuttings from plants grown under different nutritional conditions were equally sensitive to lycomarasmin injury (5).

Ludwig (9) found that the rate of flow of water through detached stems of diseased plants was slower than that through detached stems of healthy plants. The increased resistance to water flow through diseased stems was said to be due to the presence of an almost transparent gum-like material in the vessels. Such materials were not observed in fixed stem sections, but were found in free-hand sections in water mounts. His conclusion that wilting of the leaves results from such occlusions is at variance with the lycomarasmin theory.

In the present work, the mechanism of wilting was re-examined by different methods, and experiments were designed to test the validity of both the toxin and plugging theories. A preliminary report has been published (11).

MATERIALS AND METHODS.—The tomato variety Bonny Best was used in all experiments. Plants were grown in the greenhouse, usually in white silica sand and were watered with mineral nutrient solutions. In some cases they were grown in soil. Cuttings 8–12 in.

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This investigation was supported in part by grants from the Research Committee of the Graduate School, University of Wisconsin, and from the Herman Frasch Foundation.

long taken from plants 12–20 in. tall and 6–8 weeks old, were carefully selected for uniformity. Stems were cut under water with a razor blade and stem bases placed in the test solutions. For experiments requiring diseased material, plants were inoculated by removing them from the sand and dipping the intact roots in a heavy suspension of mycelium and spores. Plants were then transplanted again into sand or into a sand-soil mixture and kept under greenhouse conditions favoring disease development.

A virulent strain of *Fusarium oxysporum* f. *lycopersici* was used. The fungus was grown in 32-oz. pharmaceutical bottles, placed on the side to give greater exposure to the air. Each bottle contained 100 ml. of liquid medium. Cultures were seeded with small pieces of mycelial mat, and were grown in still cultures at 28°C. Unless otherwise indicated, the standard culture medium contained the following: NH_4NO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4 + 7H_2O$, 2.5 gm.; $FeCl_3 + 6H_2O$, 0.02 gm.; cerelose, 50 gm.; water, 1000 ml. To prepare inoculum, the culture fluids were filtered off and discarded, after which the fungus mat was washed, placed in tap water and macerated with a Waring Blendor. When filtrates were required, the cultures were filtered through celite and asbestos or were centrifuged to remove living cells.

Grieve's method (6) was used to stain the vessels and trace the vascular system of diseased and healthy plants. The tap root or stem base was severed under water and the cut end placed in 1 per cent aqueous solution of eosin. The cutting was kept under conditions favoring moderate transpiration until dye penetrated to the leaves. The petioles were then cut off ½ in. from their base and the epidermis of the stem slit with a razor blade. Next the stems were immersed for a few seconds in hot 15 per cent nitric acid solution, washed for 10 minutes in running water, and kept over night in a pan of water. After this treatment the epidermis and cortex were easily peeled off, leaving the stained xylem tissue, which could be traced readily. Another method was found useful in some cases, especially for tracing the vascular system in petioles. A basic fuchsin solution was diluted with alkaline tap water until it had a medium wine color. Tomato stems were exposed to this solution, as described above, until the dye showed faintly in the leaves. Dyed stems or petioles were placed for a few minutes in hot 85 per cent alcohol to remove the chlorophyll. The tissues were dehydrated further, first with 95 per cent, then 100 per cent alcohol, and cleared for 2 or 3 days in several changes of lactophenol. Stems and petioles prepared in this manner were colorless except for the red vascular bundles. Such preparations could be preserved for long periods in xylol.

Lycomarasmin was isolated by the method described by Plattner et al. (10). The fungus was grown for 2–4 months in the standard medium, filtered, and the residue discarded. Barium hydroxide was added to the filtrate, and the precipitate obtained was discarded. The filtrate was then evaporated to dryness *in vacuo* with a water-bath temperature of 40–45°C. The residue was dissolved in water, animal charcoal was added, the charcoal was filtered off, and methanol added to the filtrate. The precipitate which formed was dissolved in water, and the solution was brought to pH 2.6 with HCl. After addition of ethanol, the final precipitate was a fine powdery material which gave a single definite spot on a paper chromatogram.

The transpirational history of growing plants was determined by weighing of potted plants which had been transplanted into vermiculite in waxed paper boxes and sealed. Water was added to the box through a small funnel made from glass tubing. The funnel had holes in the sides which opened below the box lid and above the surface of the vermiculite, allowing any water added to flow rapidly onto the surface of the vermiculite. Another glass tube was sealed into the box for the purpose of aerating the substrate occasionally with compressed air. The entire container weighed about 300 gm. Transpiration was determined by weighing the box on a small balance (sensitivity 15 mg.) and recording the amount of water required to bring it back to the original weight. Leaf area was determined by outlining the leaves on paper and measuring with a planimeter. Plants used in each experiment were carefully selected for uniformity in size, and at the beginning of the experiment several plants were used as samples to determine leaf areas. Leaf areas of all plants used were determined at the end of each experiment.

Control of light, temperature and humidity was required in some of the experiments. A chamber (5.5 × 3.6 × 3.6 ft.) with double glass windows was fitted with 10 30-inch fluorescent bulbs. Plants were supported on a slatted grid 2 ft. below the lights. Temperature was thermostatically controlled, with cold water circulating in coils to maintain lower temperatures. Relative humidity was kept within fairly narrow limits by means of a saturated solution of calcium chloride in flat pans on shelves below the plants. Compressed air was bubbled through a saturated calcium chloride solution and fed continuously into the chamber and air was circulated within the latter by a small fan.

The sand-drip culture method described by Gallegly and Walker (1) was modified to study the effect of iron on disease development. All plants were grown at the 0.5 H concentration of Hoagland's solution, and only the quantity of iron was varied for the different treatments. Seedlings germinated in quartz sand were set in the drip-culture pots and inoculated 2 weeks later by the usual root-dip method. Several disease ratings were taken as the plants showed a progressive increase in disease symptoms. Individual plants were examined, placed in a disease class, and a disease index was determined by the method of Gallegly and Walker (1). Results were treated by analysis of variance.

EXPERIMENTAL RESULTS.—*Fungus distribution in diseased plants.*—Plants inoculated by the root-dip technique and kept under conditions favoring disease usu-

ally showed wilt first in 1 or 2 lower leaves. Other leaves on the same plants, even those directly connected by vascular bundles to wilting leaves, often appeared perfectly normal. This situation would not be expected if wilt were caused by systemic toxins. On the other hand, it suggested some local action and the presence of the fungus in the leaf petioles.

Distribution of the fungus in wilted and symptomless leaves of inoculated plants was determined by isolation from petiole fragments at selected locations. Petioles were surface sterilized and fragments plated on potato-dextrose agar. *Fusarium* isolates recovered were tested for pathogenicity. The pathogen was recovered from 56 of 60 petioles of wilted leaves. Non-wilted leaves from the same plants yielded the fungus from 36 of 60 petioles, indicating that fungus invasion of individual petioles usually preceded expression of symptoms in the leaf.

The possibility of internal spread of the fungus by spores in the transpiration stream was suggested by the wide distribution of the fungus in inoculated plants. Inoculum for the experiment was obtained by growing the pathogen in shake culture, where most growth is in the form of bud cells, indistinguishable from microconidia. Mycelial fragments were removed by filtering cultures through coarse filter paper which allowed the bud cells to pass through. The resulting suspension was washed by centrifugation and adjusted to 50,000 cells per ml. Tomato cuttings 7 in. long were placed in the suspension and allowed to take up the bud cells with the transpiration stream for several hours. The distribution of the fungus in these cuttings was then determined without further delay by plating stem and petiole fragments on agar. The pathogen was found throughout the lower half of the stem. It was often recovered as far as 6 in. up the stem and in the bases of larger petioles. When such cuttings were kept under conditions favoring disease development, wilt appeared in 10–15 days. Cuttings inoculated in this way obviously had many loci of infection. Movement of microconidia in vessels is thus considered possible, although distribution by mycelial growth no doubt occurs as well.

Vascular reactions of diseased plants.—A) *Plugging.*—Vessel plugging in diseased plants was determined by dye injection of the vascular system as described under Materials and Methods. Eosin-injected plants were treated by Grieve's technique (6) or were examined by free-hand sections for dye distribution. Fuschin-injected plants were cleared with hot alcohol followed by lactophenol; this method proved to be especially good for petioles. Non-diseased plant materials, treated at the same time, were used as controls in all cases.

Twenty-five diseased plants were injected with eosin by placing the severed stem base in the dye solution and allowing the transpirational pull to distribute the dye through the vessels. Microscopic examination of free-hand sections showed that many vessels in the stems were not conducting the dye, and that the petioles of completely wilted leaves were invariably free of dye. Unilaterally wilted leaves showed the dye in the large petiole trace on the non-wilted side, while the large trace on the wilted side and the median trace failed to conduct dye. The petioles of non-wilted leaves from the same plants showed the dye in all bundles, while leaves showing slight flaccidity had some vessels in petiole bundles which conducted dye. Included in these experiments were plants showing all gradations from very slight to very severe wilting. In every case, leaf wilt was associated with failure of the petiole bundles to conduct dye. Also, every wilting plant had bundles in the stem which failed to conduct, the number being correlated with stage of disease development.

Dyes were drawn through diseased stem segments to determine whether failure of vessels to conduct was due to plugging or to decreased transpiration in wilting leaves. The apical ends of the stem segments were attached by rubber tubing to a vacuum pump, and the basal ends were placed in an eosin solution. Diseased and healthy stems were hooked in parallel, and suction was applied until the healthy stems were thoroughly permeated with dye. Ten diseased stem segments, 6–10 in. long, were used. Microscopic examination of free-hand sections showed plugging in all diseased stems, the extent of plugging being correlated with the severity of disease. Badly wilted plants had most vessels plugged, plants in early stages of wilt had few stem vessels plugged, and heathy plants showed the dye in all vessels.

Plugging in petioles was studied further by injecting dye through the petioles into plants *in situ*. It is well known that tension created by transpiration will draw solutions into the plant through severed petioles. Dye injected in this way through healthy petioles was soon evident in the vessels of the stem. When petioles of wilting leaves were similarly exposed, no dye was drawn into the stem, even after several hours' exposure under conditions favoring high transpiration. When unilaterally wilted leaves were used, and free-hand sections cut from the base of the petiole, only the large petiole trace on the non-wilted side showed the dye, while the median trace and the large lateral on the wilted side were evidently plugged.

Dye distribution in diseased stems was studied further by Grieve's technique (6). Ten plants were injected by allowing transpiring cuttings to take up the dye, and in 5 plants the dye was forced through stem segments as described above. Two transpiring plants were injected simultaneously from the basal end and from the apical end, the latter being accomplished by fitting a small reservoir of dye with a rubber tube to the top of the stem. Inspection showed essentially the same distribution of dye found by examining free-hand sections, with the additional fact that vascular bundles could be traced through their whole length, and the point of plugging could be determined. It was found that dye was conducted into the bases of wilting petioles in some cases, while in other cases the traces leading to petioles were plugged one or more

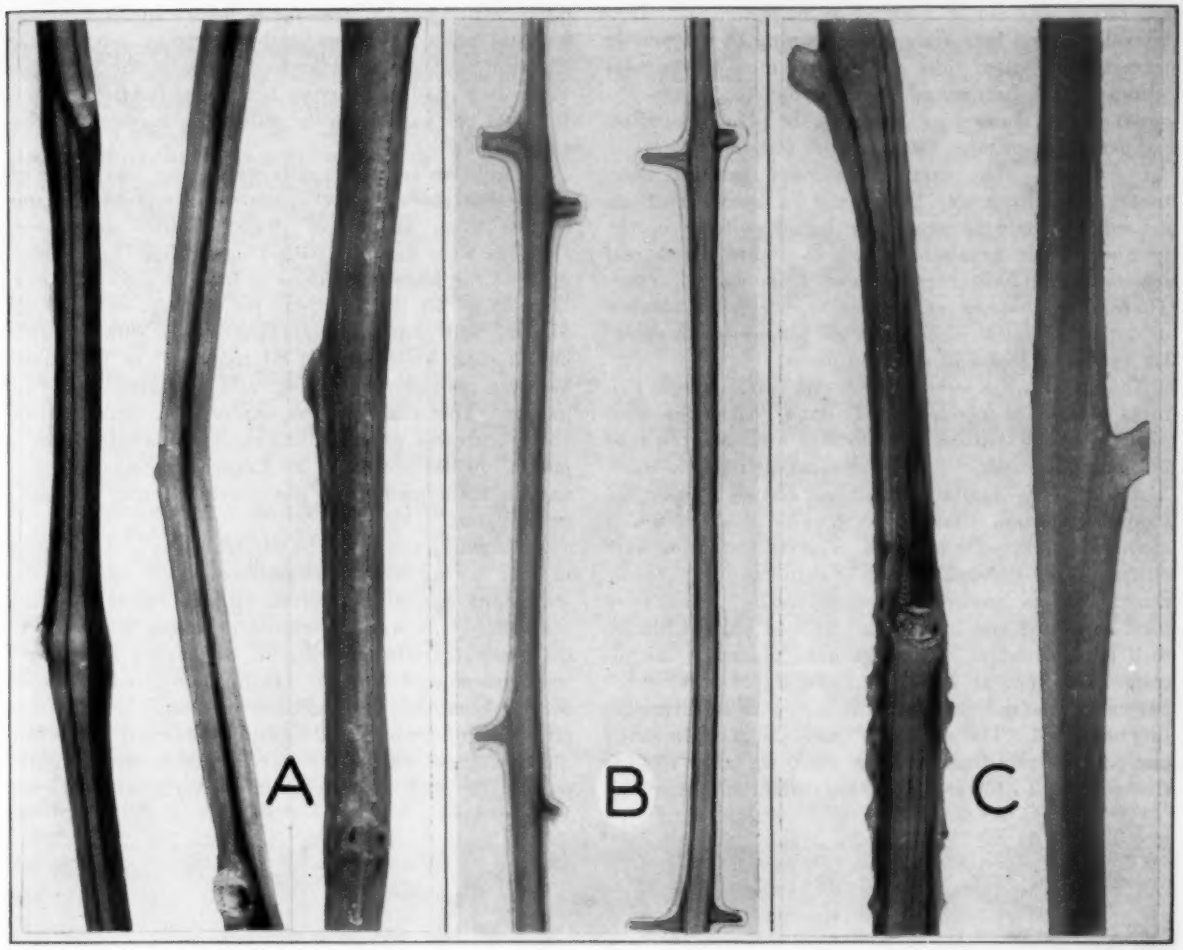

FIG. 1. A) Dye distribution in infected and healthy stems when cut ends were placed in eosin solution and the epidermis and cortex removed after treatment. Left, healthy stem with all bundles dyed; center, moderately diseased stem with some bundles dyed; right, severely diseased stem with no bundles dyed. B) Cut petioles of wilted and healthy leaves exposed to dye. Left, petiole from wilting leaf showing vascular browning but no dye; right, petiole of healthy leaf with all bundles dyed. C) Stems, cut ends of which were exposed to filtrates from replacement cultures and then cleared with hot alcohol and lactophenol. Left, treated with unheated filtrate showing vascular browning; right, treated with heated filtrate showing no vascular discoloration.

nodes down the stem. Individual bundles did not have a sharp demarcation point for the dye, but the color gradually faded out, indicating that all vessels in the bundle were not plugged at the same point. All bundles in healthy plants showed the dye very distinctly throughout (Fig. 1, A).

Petioles and stems, vessels of which were stained with basic fuchsin, showed the same distribution of dye. When fuchsin was used, chlorophyll was removed with hot alcohol, and tissues were further cleared with lactophenol. This method was found to be especially useful to illustrate plugging, browning, and dye conduction in petioles (Fig. 1, B).

Some idea of the nature of plugging was obtained by long exposures to the dye solutions. When moderately diseased cuttings were exposed to eosin under conditions of relatively high transpiration for 24 hours, the dye became generally distributed throughout infected bundles. This would be expected if conduction through vessels were hampered by vessel fluids more viscous than normal.

B) *Browning.*—One of the characteristic symptoms of *Fusarium* wilt is browning of the vascular bundles. This condition to a greater or lesser extent was invariably found to be associated with disease. Free-hand sections cut across diseased stems and examined microscopically showed dark pigmentation in the walls of infected vessels and in the walls of xylem parenchyma cells surrounding infected vessels. Walls of infected vessels often appeared swollen and corroded.

Petioles of wilted leaves were examined for vascular browning. The diseased material was treated with hot alcohol to remove chlorophyll, followed by lactophenol treatment for 2 days to clear the tissues. Examination showed some degree of browning always present in petioles of wilted leaves, although the dis-

coloration was not so marked as in the stems. Unilaterally wilted leaves showed browning of the petiole trace on the wilted side, and no browning on the non-wilted side. Non-wilted leaves from the same diseased plants showed no browning in petiole bundles.

Effects of culture filtrates and polysaccharides.—A) *Filtrates.*—The original evidence for the toxin theory of wilting was the injury to tomato cuttings caused by crude filtrates from liquid cultures of the fungus. Toxic metabolites such as organic acids and lycomarasmin have been isolated from such filtrates. Therefore it became of interest to determine whether or not the filtrates could cause the plugging and vascular browning found in diseased plants.

Tomato cuttings were treated with filtrates from cultures varying in age from 4–75 days. Since the composition of the culture medium may influence the type of metabolic product formed, several media were used, including the standard medium, Czapek's solution, Czapek's solution diluted with 4 parts of water, and a medium with organic nitrogen. The culture fluids were either filtered through a layer of celite and asbestos or centrifuged to remove all fungus cells. They were used undiluted and in dilutions up to 1 part of filtrate to 8 parts of water. Cuttings were placed in the filtrates and kept at 27°–28°C. and approximately 50 per cent relative humidity until moderate to severe injury occurred. The ability to cause plugging in stems and petioles was determined by the eosin test. Results showed that all filtrates used, regardless of age or medium, which caused leaf blade injury in 24 hours or less did not cause vascular browning or plugging at that time. This rapid leaf response is the symptom most often used in assaying toxicity of fungus filtrates. It is not comparable to the wilting as it occurs in diseased plants.

In addition to the rapidly appearing leaf-blade injury other more slowly appearing symptoms became evident when filtrates of young cultures were used. Cuttings were kept for 3 to 4 days, with two changes daily of the filtrate solutions to prevent growth of contaminants. By the third day the cuttings in undiluted filtrates had generally collapsed with a soft rot without vascular browning. In dilutions of 1 to 3 or more, however, vascular browning and plugging were apparent. This reaction was observed in filtrates from young cultures only. Filtrates from cultures over a month old did not cause it. Control cuttings in water and in fresh media did not show vascular browning or plugging.

An attempt was made to eliminate some of the more rapidly acting toxic metabolites which affected the leaves and possibly interfered with the vessel-browning reaction. This was considered desirable since practically any material in solution will cause leaf injury when introduced into the transpiration stream. In order to eliminate accumulated metabolic products, a technique of replacement cultures was used. The fungus was grown in the standard liquid medium in still culture at 28°C. until a dense mycelial mat was

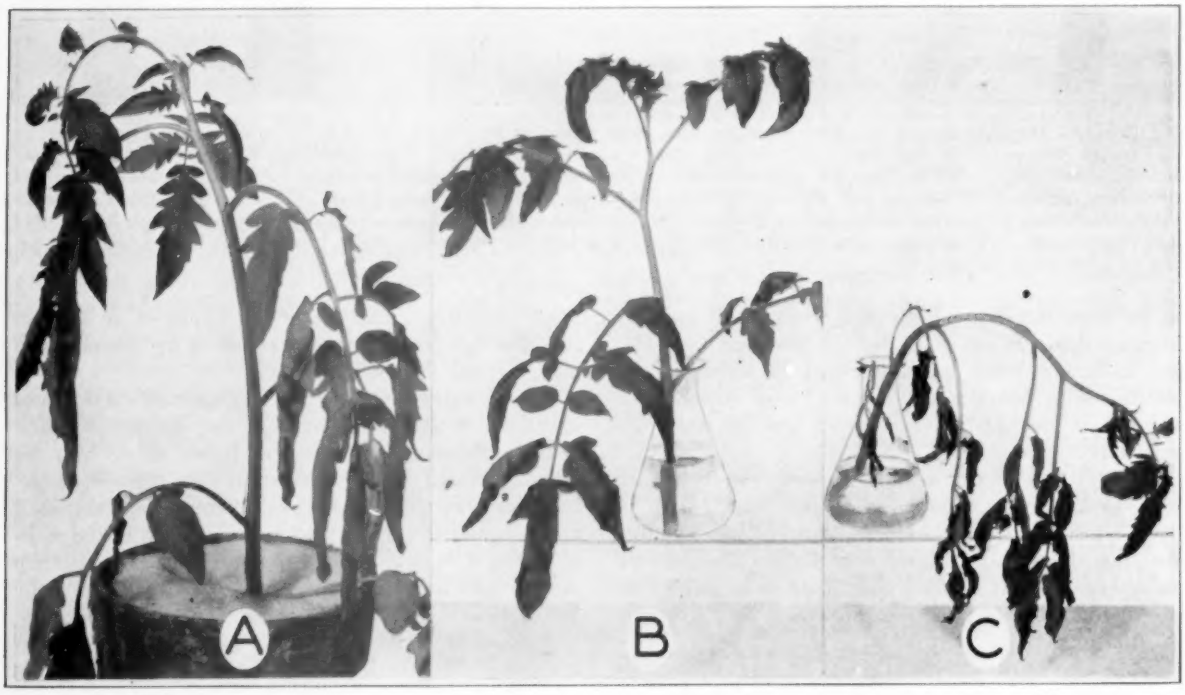

FIG. 2. A) Symptoms typical of plants inoculated in the greenhouse by removing them from the sand-nutrient substrate, dipping the roots in a suspension of fungus mycelium and spores, and transplanting to the original substrate. B) Tomato cutting 48 hrs. after insertion in filtrate from replacement culture which had been boiled for 10 minutes. C) Cutting 48 hrs. after insertion in unheated portion of the same filtrate.

formed. The 8- to 20-days-old cultures were filtered, the filtrates were discarded, and the mycelial mats were washed and the excess water removed by suction in a Büchner funnel. The washed mycelial mat was next suspended in its original volume of Czapek's solution diluted 1:4 with water. After 24–28 hours in the fresh medium, the cultures were filtered and the filtrate used to treat cuttings in the usual manner.

Many tomato cuttings from plants of various ages were exposed to the replacement culture filtrates. Greenhouse conditions and controlled conditions were used. The test solutions were changed twice daily to eliminate growth of contaminants, and the stem bases were trimmed daily to eliminate possible vessel plugging at this point. Cuttings usually began to wilt in about 24 hours, and after 2 days were moderately to severely wilted. Some leaf blade injury occurred, but symptoms consisted chiefly of flagging of petioles and upper stems (Fig. 2, C). At this stage eosin could not be forced through the vascular system, indicating plugging. Peeling back the epidermis and cortex revealed vascular browning beginning in some spots. After 3 or 4 days the vascular browning was very marked (Fig. 1, C). These symptoms were comparable to those in severely diseased plants, especially when disease development had been rapid.

A few preliminary experiments were conducted to characterize the active material in the replacement filtrates. The vessel browning and plugging factor was found to be heat-labile, most of its activity being destroyed after 10 minutes at 60° C. (Fig. 1, C). The activity was not lost after dialyzing for 15 hours. Further characterization will be reported in a later paper.

B) *Polysaccharides.*—The injurious effect of polysaccharides on tomato cuttings is well known (7). It has been suggested that these materials are responsible for at least 10 per cent of the symptoms of tomato wilt (4). Two types of injury occur, apparently depending on the size of the molecule. Those polysaccharides with molecular weights of 1000 to 5000 cause injury to the leaf blade, while those with molecular weights above 5000 cause flaccidity of stems and petioles (7).

The effects of polysaccharides on the xylem of cuttings were examined, using inulin as an example of those causing leaf injury and xylan and agar as examples of those causing wilt. They were used in dilutions (.05 to 0.2 per cent solutions or suspensions) which caused moderate to severe injury in 24 hours, and the ability to cause plugging was determined by the dye test. Results showed that inulin caused no blocking of vessels in stems or petioles, even when leaf blades were severely injured. The most dilute xylan and agar suspensions used caused blocking of the vessels near the base of the stem, resulting in flagging of the whole cutting. When 1 in. of the stem base was trimmed off before the cutting was exposed to the dye, all vessels became well stained. When such trimmed cuttings were placed in fresh water they recovered unless wilting had been very severe.

The similarity of the polysaccharide-induced injury to *Fusarium* wilt is questionable. Neither inulin, xylan, nor agar caused vascular browning, and inulin did not cause plugging in the stems or petioles. Xylan and agar did cause blocking of the vessels, but this effect apparently resulted from an increase in the viscosity of the fluid, which was not readily conducted through the small vessels. Only in this respect might wilt caused by larger polysaccharides resemble wilt as it occurs in infected plants.

The Role of lycomarasmin.—A) *Comparative effects of Fusarium and lycomarasmin.*—The apparent significance of vascular plugging and browning in the syndrome of *Fusarium* wilt offers a tool for the critical evaluation of lycomarasmin and other toxins. Accordingly, lycomarasmin was isolated by the method described by Plattner et al. (10) and tomato cuttings were exposed to it in the usual manner. A solution was used which caused moderate to severe leaf injury in 24 hours. After 24 and 48 hours, cuttings were given eosin dye tests to determine plugging. The toxin caused injury to leaf blades, but no plugging or browning in the vascular elements. The internal effects of lycomarasmin therefore are not the same as the internal effects of *F. oxysporum* f. *lycopersici.*

External symptoms of *Fusarium* wilt and lycomarasmin injury differed in kind. Inoculated plants kept under conditions favoring rapid disease development first showed flaccidity of leaves and petioles (Fig. 2, A) without necrosis of leaf blades. Wilt usually began in a lower leaf and gradually, over a period of several days, the whole plant became affected. In early stages the leaves often wilted badly during the day and recovered at night when transpiration was low. Leaf yellowing was common in older diseased plants, in later stages of disease, and under conditions which favored slow disease development. Leaf-blade injury followed wilting, much as in wilt induced by lack of water, and only rarely did necrotic spots appear on the leaves before wilting occurred. Other symptoms observed were epinasty of petioles and stimulation of rootlets along the stem, both of which have been described previously by others. Lycomarasmin, on the other hand, caused first a necrosis of leaf tips and margins, gradually involving the whole leaf blade without causing flaccidity of the petioles. Lycomarasmin injury was irreversible; the leaves did not recover when placed under conditions of low transpiration.

B) *Effects of lycomarasmin and Fusarium on transpiration.*—Lycomarasmin is known to have a striking effect on the water relations of tomato cuttings. When used in the proper dilution, it causes first a shock period of decreased transpiration, then a period of excessive water loss, and finally wilt. Cuttings exposed to more dilute solutions did not wilt, but water loss was increased (3). If lycomarasmin is produced within the diseased plant, and if it is important as the wilting agent, then diseased plants should show excessive water loss before wilting occurs.

The lycomarasmin preparation used in the present

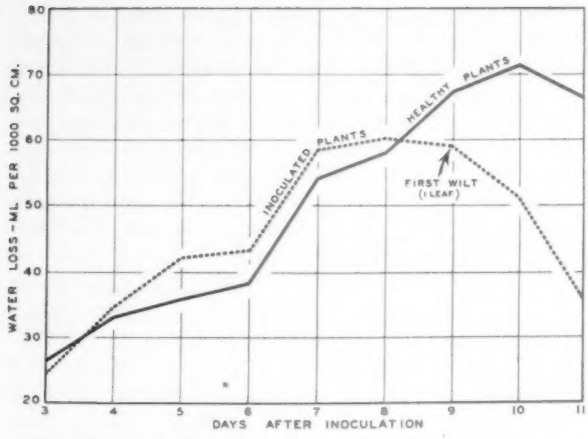

FIG. 3. Transpiration curves based on the averages of values from 5 diseased and 5 healthy plants held at 28° C. and 40–44 per cent relative humidity.

experiments was tested for its effect on transpiration of tomato cuttings. Humidity, temperature, and light were controlled, and cuttings were held until transpiration was stabilized before the lycomarasmin solution was added. Transpiration was determined by hourly weighings of the cuttings and solutions. The typical transpiration curve described by Gäumann (3), with a shock phase and a phase of high water loss, was obtained.

Water loss by diseased plants was compared with water loss by healthy plants under controlled conditions and under greenhouse conditions. Plants were inoculated in the usual way and roots were sealed in waxed paper boxes as described in Materials and Methods. Under controlled conditions (28° C., 40–50 per cent relative humidity) the transpiration of 5 inoculated and 5 noninoculated plants was compared in each of 3 experiments. Under greenhouse conditions (28°C. ± 2°) the transpiration of 10 inoculated plants was compared with the transpiration of 10 healthy plants in 3 experiments. In 4 experiments, weighings to determine transpiration were taken twice daily, but in 1 greenhouse experiment and in 1 controlled experiment, weighings were made every 2 hours during the light period, when transpiration was high, to detect any possible peaks or recessions in the transpirational curve. Leaf areas were taken at the end of each experiment, and results recorded as the water loss per 1000 sq. cm. of leaf surface.

Results of a typical experiment are illustrated in Figure 3. In every experiment, transpiration of diseased plants decreased preceding and during wilting. The average transpiration values did not give an entirely true picture, however, since disease did not progress at the same rate in all plants, and some individuals wilted several days before others showed symptoms. When each plant was considered separately, transpiration decreased without exception immediately preceding and during wilting, and never increased after wilting started. Transpiration curves of individual plants showed no abnormal peaks or depressions, even when weighings were made every 2 hours.

Gradually decreased transpiration would be expected if it is assumed that more and more vessels become plugged as the disease progresses. However, plugging is evidently more drastic than transpirational data indicates, since as much as 28 per cent of the leaf area of a healthy plant was removed without a significant decrease in transpiration.

Inoculated plants showed slightly higher average transpiration than healthy plants immediately after inoculation (Fig. 3). This slight difference was observed in all experiments, but it was statistically significant in only one. The explanation of this phenomenon is not known. Lycomarasmin probably is not involved, since the increase occurs as early as 2 days after inoculation, and lycomarasmin has been found in quantity only in old cultures. Kramer (8) has shown a similar response in plants injured by flooding the soil.

C) *Effect of iron deficiency on disease.*—The toxicity of lycomarasmin is affected by the presence of iron. Gäumann (5) has shown that lycomarasmin is fully 10 times more toxic to tomato cuttings if free iron is present in the toxic solution. Therefore if lycomarasmin is the important wilting agent in diseased plants, inoculated plants containing a normal amount of iron should wilt more rapidly than inoculated iron-deficient plants.

The general methods used in 2 experiments to determine the effect of iron on disease development are described under Methods and Materials. The design for the first experiment was a randomized block with 3 replications, 4 pots per replication and 10 plants per pot. The following treatments were used:

1) To remove any traces of iron the sand used in this case was washed with concentrated hydrochloric acid and thoroughly rinsed before the seedlings were set. All nutrient solutions were adjusted to pH 6.5 to precipitate any iron impurities.

2) No iron was given after inoculation, but 0.5 p.p.m. of ferric chloride was supplied in the nutrient solution before inoculation. At inoculation time, sand was washed with concentrated hydrochloric acid, rinsed with distilled water and neutralized before plants were reset. Nutrient solutions were adjusted to pH 6.5 after inoculation.

3) $FeCl_3$, 0.5 p.p.m., was supplied in the nutrient solution before and after inoculation. Sand was not washed with acid, and the nutrient solutions were pH 5.3.

4) The same as (3) except that nutrient solutions contained 1.0 p.p.m. of ferric chloride.

Plants showed distinct growth responses to the treatments. Those given Treatment 1 were stunted and showed severe iron deficiency symptoms at the time of inoculation. At the end of the experiment, plants given this treatment were about ¼ the size of plants in the other treatments, and were stiff, woody and pale

yellow. Plants in all other treatments appeared normal at the time of inoculation. Plants given Treatment 2 showed the first signs of iron deficiency in new leaves 3 days after inoculation, and developed a striking iron deficiency before disease symptoms began. These plants, however, were almost equal in size to plants in the other treatments, and they developed enough new foliage after inoculation so that the majority of the leaves were showing iron deficiency symptoms when wilting began. Plants in treatments 3 and 4 showed no differences in growth responses.

The first disease reading was taken 2 weeks after inoculation, and 2 other readings were taken as symptoms became more severe. Statistical analysis of disease indices showed no significant difference between plants given no iron after inoculation and those plants given normal and excess iron. Only plants which were given no iron during the whole experiment (Treatment 1) had lower disease indices. These plants, however, had long since ceased to grow and appeared stiff and woody. Their disease indices were not comparable to indices for the other treatments.

In a second experiment disease development in iron deficient and in normal plants was compared at 2 pH levels. Sand cultures were again used, and all plants were watered with 0.5H solutions until the time of inoculation. At inoculation time, plants scheduled to receive no iron were transplanted into acid-washed sand, and thereafter watered with 0.5H solution without iron. In an experimental design of 5 replications with 10 plants per replication, the following treatments were used.

1) Normal iron (0.5 p.p.m. of ferric chloride) was given in nutrient solutions at pH 5.3.
2) No iron was added to nutrient solution after inoculation. pH of solution was 5.3.
3) Same as 1) except pH was 6.5.
4) Same as 2) except pH was 6.5.

Results showed no differences between disease indices of iron deficient and normal plants at either pH level. Figure 4 illustrates the close correlation obtained with nutrient solutions at pH 5.3. A further comparison was made of wilt in top leaves, since this was the area which showed the striking deficiency symptoms. Again there were no differences between iron deficient and normal plants. It is concluded, therefore, that under the conditions of these experiments iron nutrition did not affect disease development.

Discussion.—The results of this investigation show that the disease syndrome of *Fusarium* wilt of tomato is initiated by interference with the natural upward flow of water and nutrients through the vascular system. It has been demonstrated that decline of transpiration of infected plants begins shortly before signs of disease appear and continues steadily during the remainder of the disease cycle. Study of eosin transport shows that by the time disease symptoms appear the xylem traces give signs of being plugged and that the degree of plugging is sufficient to account for the decline in transpiration. Unilateral yellowing and

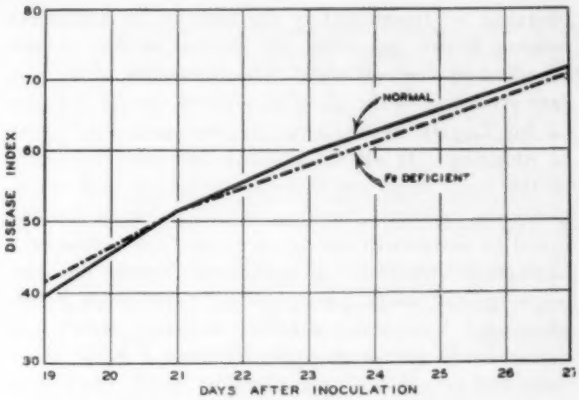

Fig. 4. Wilt development in inoculated tomato plants grown in sand watered with a normal balanced nutrient solution and with one without iron, both at pH 5.3.

wilting of leaves and leaflets can be correlated rather closely with the differential plugging of traces and the latter in turn may be accounted for by random distribution of infectious mycelia in the vascular system.

Whereas previous investigations have implied that disease symptoms are brought about by toxic materials being transported in advance of the pathogen, present studies of the distribution of the pathogen in diseased plants does not bear out such a conclusion. When cut stems were inserted in suspensions of bud cells or microconidia there followed a widespread distribution of the spores within a few hours, and many separate loci of infection followed. When a plant becomes naturally infected through the root system and microconidia are produced in the tracheae they can, therefore, be distributed in the transpirational stream. However, when any given trace becomes plugged further transport of fungus or toxic materials secreted by it is greatly reduced if not entirely stopped in that trace.

Browning of vascular bundles, principally in the thick walls of xylem elements, is an important part of the disease syndrome. It might be the result of toxic materials excreted by the pathogen or, since it appears to be closely associated with the presence of the fungus, it might be the result of action of exoenzymes of the latter. Filtrates from cultures of the pathogen 4 weeks old or more, while they do cause some atypical wilting of the leaflets of stem cuttings, do not cause plugging and do not bring about vascular browning. However, when filtrates of young cultures are used in the proper dilutions, or when filtrates from replacement cultures are used, browning and vessel plugging are caused in cut stems. It is obvious then that materials excreted by the mycelium within a very short time in replacement cultures can account for the main pathological features of tomato wilt. While the nature of this action has not been determined it suggests one of enzymic action on cell wall materials. This action apparently releases substances capable of

plugging the traces and by the same or by a different process brown pigments are formed within or are adsorbed by the cell wall. The remainder of the disease syndrome of the plant as a whole can be accounted for largely by the rate, degree, and distribution of plugging. If all or most of the xylem elements of the main stem are infected uniformly and simultaneously, general plugging would naturally be followed by severe wilt and rapid death. Continuous and haphazard infection of occasional xylem elements might permit more microconidial distribution before plugging. With many noninfected traces, which condition would permit more nearly normal water movement and growth for a considerable period, the disease syndrome would be expected to develop much more slowly. In fact the last type of disease picture is the one which is most commonly seen in nature.

It is obvious that the interpretation of the experimental findings presented is not in accord with the lycomarasmin theory of causation of wilt propounded by Gäumann and associates (2, 3, 4, 5, 10). There are several reasons for disagreeing with that theory. Lycomarasmin is produced in quantity in liquid medium cultures only after 2–3 months while the fungus in the infected plants induces wilt within 10 days. A highly virulent strain of the pathogen was found by them to produce much less lycomarasmin than a very weakly pathogenic strain. Although greater wilt production is indueed by lycomarasmin combined with iron there was no such effect produced when infected plants were fed increasing amounts of iron. Lycomarasmin produced in liquid culture, while it induces atypical wilting of leaflet lamina of stem cuttings, does not induce plugging and does not cause vascular browning. The transpirational curves of stem cuttings inserted in solutions of lycomarasmin are distinct from those recorded in infected plants.

While the writers conclude that the primary cause of the wilt disease syndrome is plugging of the xylem elements they do not hold that toxic excretory products of the fungus are not important. They do not exclude the possibility of secondary causal factors which bring about some of the symptoms such as vein-clearing and epinasty. It is clear, however, that the excretory products concerned are produced promptly in replacement cultures and either are not found or are entirely obscured in old cultures. Moreover the effect of the excretory products are local and function by bringing about plugging after which, in the xylem element concerned, advance of the fungus and diffusion of fungus excretory products are greatly reduced or entirely stopped. The concept of the wilt disease resulting from a general systemic toxemia does not appear to the writers to be justified, and certainly it is not supported by the experimental facts at hand.

SUMMARY

Fusarium oxysporum f. *lycopersici* was found to cause significant responses in the vascular systems of tomato plants preceding or accompanying external symptoms. The fungus was generally present in petioles of wilting leaves, and was often present in leaves before they showed symptoms. The comparative conducting ability of diseased and healthy plants was determined by placing roots or stem bases of transpiring plants in dye solutions until vessels of healthy plants were well stained. Microscopic examination of free hand sections from the stems and petioles showed that many vessels in diseased plants did not conduct the dye. The same results were obtained when dyes were drawn through segments of stems and petioles. Diseased plants were also examined by clearing the stems and petioles by various techniques, and studying the dye distribution in the vascular bundles. Evidence obtained from plants in all stages of wilting indicates that vessel blocking is the immediate cause of wilt. Plugging of vessels in petioles appears to be especially significant.

The effects of culture filtrates on the vascular systems of tomato cuttings were determined. Crude culture filtrates from cultures of all ages which caused leaf blade injury in 24 hours or less did not cause vascular plugging or browning. When diluted filtrates from young cultures were used, and when cuttings were held for 3–4 days, some plugging and vascular browning appeared. Filtrates of replacement cultures caused vascular browning and plugging with little of the leaf blade injury caused by other filtrates. Preliminary characterization showed that the active component was heat labile and non-dialyzable.

Polysaccharides with small molecules, which cause leaf blade injury, did not cause vascular plugging or browning. Dilute suspensions of polysaccharides with large molecules did cause vascular plugging but no browning. The plugging effect is believed to be due to increased viscosity of the fluids.

Several lines of evidence against lycomarasmin as the principal wilting agent were obtained. Lycomarasmin did not cause the vascular plugging and browning characteristic of diseased plants nor did it cause typical external symptoms of *Fusarium* wilt. If lycomarasmin is the chief cause of wilt, increased water loss would be expected immediately preceding or accompanying wilting. Instead, transpirational history of diseased plants showed a progressive decline in water loss as disease developed. The high toxicity of lycomarasmin to tomato cuttings depends on the presence of iron. There was, however, no difference in disease development in inoculated iron-deficient plants and normal plants.

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COPPER SULPHATE FOR THE CONTROL OF DAMPING-OFF IN TOBACCO SEEDBEDS [1]

Rodrigo G. Orellana [2]

Seedbeds of Virginia and Burley varieties of tobacco (*Nicotiana tabacum* L.) suffer severe losses from damping-off in the rainy season under the tropical conditions of northern Venezuela. When emergence is severely curtailed by damping-off, bare, circular spots, varying from a few cm. to 40–50 cm. in diameter, appear on the seedbed (Fig. 1, a); they enlarge rapidly. Spread of damping-off, however, may be checked in non-crowded beds when the weather becomes warm and dry.

Symptoms of damping-off occur near or at soil level. Destruction before emergence of seedlings from the soil is due to attack by pathogenic fungi or to seed decay when the soil conditions for germination are unfavorable. Damping-off on emerged seedlings causes discoloration, disintegration and collapse of stems. Symptoms of damping-off of tobacco in Venezuela are comparable to those occurring elsewhere (1). This paper lists the organisms found associated with damping-off in Venezuela and describes attempts to control the disease by application of copper sulphate to the seedbed.

PARASITES INVOLVED.—Isolations from infected seedlings and from seeds that failed to emerge in naturally infested soil yielded mostly colonies of *Rhizoctonia* (*Corticium*) *solani*, *Pythium* spp., and *Fusarium* spp. In a few instances *Sclerotium* spp. were also isolated from infected tobacco seedlings. *Pythium aphanidermatum* has been reported attacking tobacco in Venezuela (2), although the type and extent of damage has not been indicated.

Soil in wooden flats was artificially infested with isolates of *Rhizoctonia solani* or with isolates of *Pythium* spp. obtained from diseased tobacco seedlings, and then sown to tobacco. Typical symptoms of damping-off appeared on plants, on the seed prior to its emergence, and on emerged seedlings. Both fungi were pathogenic and were easily re-isolated from damped-off seedlings.

Natural incidence of damping-off was high when tobacco seedlings had very succulent stems. This pronounced succulence occurred when the microclimatic zone beneath the seedling foliage was high in humidity and when the soil of the seedbed remained constantly wet, as in over-crowded tobacco stands. Even after the climate above the seedbed became warm and dry, the high humidity persisted under the foliage and in the soil of densely planted beds. In the natural infections observed there was no evidence of stem injuries that could have provided a path for penetration of damping-off organisms. This observation partially corroborates that of Johnson (4) who stated that varieties and even strains of a single species of plants vary greatly in rate of water congestion under the same environmental conditions, a fact which predisposes the plant to infection by certain bacterial and fungal parasites, without relation to wounded tissue.

CONTROL.—Tobacco growers in Venezuela have attempted to control damping-off by burning the soil or by applying either coarsely ground copper sulphate or Bordeaux mixture directly to the seedbed (2). Since these methods proved only partially satisfactory, it was decided to study copper sulphate in solution for its effects on damping-off in tobacco seedbeds and on the development of tobacco plants.

Copper, according to Steinberg (5), is required at certain concentrations by some fungi pathogenic to tobacco, namely, *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium irregulare*, *Cercospora nicotianae*, and *Thielavia basicola*. In a preliminary investigation it was found that 0.01 gm. of cop-

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FIG. 1. a) Natural incidence of damping-off of Yellow Special tobacco in the seedbed. b) Seedbed treated with copper sulphate at the rate of 30 gm. per sq.m. showing control of the disease. c) Seedbed treated with copper sulphate at the rate of 50 gm. per sq.m. showing reduced emergence and control of damping-off.

per sulphate per 100 c.c. of malt extract-agar medium stimulated growth of a *Fusarium* sp., as indicated by size of the colony. Similar stimulation was observed with *Pythium* sp. on beef extract-agar medium containing the same amount of copper sulphate. At 0.2 gm. of copper sulphate per 100 c.c. of the same culture media the development of these 2 fungi was, however, completely suppressed.

In order to test the effect of copper sulphate solutions on the control of damping-off of tobacco, a series of seedbed treatments was made at the experimental farm, Gonzalito, in northern Venezuela, during the rainy months of the year, July to October. The soil used was a clay loam with pH of approximately 6.8. The level of the seedbeds was raised 35 cm. by embanking the soil. Two, 20, 30, 50, and 100 gm. of copper sulphate per square meter were added in aqueous solution to seedbeds, replicated 5 times. The treated beds were then raked to a depth of approximately 10 cm., and the seedbed was sown immediately with U. S. certified seed of Yellow Special tobacco, a Virginia variety. The seedbeds were covered with cloth or gamalote straw which was removed daily for 1 or 2 hours when the weather permitted.

Germination percentages (Fig. 2) for the 5 replicates were estimated on the twelfth day after treatment and sowing, on the basis of the number of seedlings emerging in 100 sq. cm. of seedbed. Germination was fairly uniform in all beds receiving 30 gm. or less of copper sulphate per sq.m. Higher rates of treatment reduced germination (Fig. 1, c).

The effect of these levels of copper sulphate in the control of damping-off tobacco (Fig. 1) was striking. At a concentration of 20 gm. of copper sulphate per sq.m., however, the disease incidence increased after the third month. A similar increase was not observed in the checks nor with any of the other rates, including the 2-gm. rate. Most of these plants at the 20-gm. rate were chlorotic and had brown, sunken lesions on the base of the stem. *Rhizoctonia solani* and *Fusarium* spp. were isolated from these infected stems. The 20-gm. rate, although promising, affords only temporary

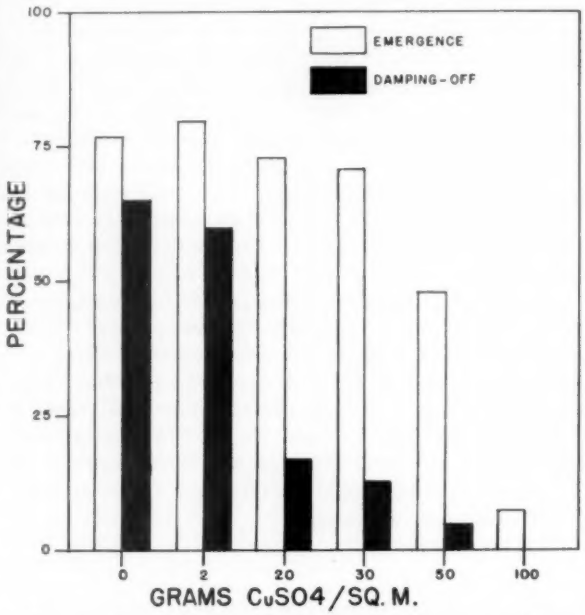

FIG. 2. Comparative effect of 5 rates of copper sulphate applied in aqueous solution directly to the seedbed on the seedling emergence 12 days after sowing and on natural incidence of damping-off of Yellow Special tobacco seedlings 3 months old in a clay loam soil of pH 6.8 during the rainy season, at Genzalito, Venezuela.

control of damping-off of tobacco. The 30-gm. rate was superior in its effectiveness to the 20-gm. rate because, while germination was only 2 per cent less than at the 20-gm. rate (Fig. 1, b), damping-off incidence was 8.8 per cent less. Also, there was no further spread of disease at the 30-gm. rate. Perhaps the low incidence of the disease at 50 and 100 gm. of copper sulphate per sq.m. was partly due to the non-crowded condition of the seedbed as a result of decreased germination.

Copper applied to soils of low acidity is known to be toxic to plants. In minute amounts, however, copper is essential for normal plant development, and it may correct conditions caused by copper deficiency. The value of copper treatment is also known to be modified by soil factors which influence copper solubility, namely, pH and adsorptive capacity (3). The availability of copper tends to decrease with increasing pH (6).

Plants grown on copper sulphate treated beds were examined for copper toxicity. The most conspicuous symptoms were dwarfing of the plant and thickening, curling and narrowing of the leaf blade. An opaque, white-green cast was also observed on plants grown at the 2 higher levels of copper.

At 2, 20, and 30 gm. of copper sulphate per sq.m. the plants appeared normal. Except for a few plants that had toxicity symptoms, no other effect of this substance as applied to the soil could be detected visually. The few plants that had symptoms of malformation at the 20 and 30 gm. copper levels were possibly those that grew in spots where the copper sulphate solution was more concentrated. At the 20-gm. rate chlorosis appeared after the third month. This chlorotic symptom might have been due to the lesions that developed on the stems. Root development of plants grown at 30 gm. of copper sulphate per sq.m. appeared to have been stimulated, as was seen by comparing them with roots of plants from the other treatments and by their greater mass.

The toxicity symptoms caused by copper sulphate were very conspicuous on plants grown at 50 and 100 gm. per sq.m. Apart from the symptoms on the aerial plant parts, primary and secondary roots were underdeveloped.

Tobacco plants that grew in the control beds and in the beds treated with 30, 50, and 100 gm. of copper sulphate per sq. m. were transplanted when 3 months old to a site where copper had not been applied, in order to observe the effect of these treatments on the adult plants. Transplants from the 30 and 50 gm. treatment beds developed normally; considerable stunting remained evident on transplants from the 100 gm. copper treatment beds. Chlorosis was an obvious symptom on the adult plants of the control beds after transplanting, whereas the plants that came from the copper treated beds had no such symptoms. As there were no lesions on the stems, this may have indicated a copper deficiency in the checks.

Since the effectiveness of copper sulphate for control of damping-off of tobacco depends not only on the rate of application but on the soils used, further investigations should be made regarding the adsorptive capacity of different soils for copper and the effect of copper sulphate on the tobacco plant and the curing processes used for the leaf.

SUMMARY

Damping-off of Virginia and Burley tobacco causes severe losses in seedbeds in Venezuela, particularly in the rainy season. Control of the disease was attempted by applying aqueous solutions of copper sulphate to the soil at the rates of 2, 20, 30, 50 and 100 gm. per sq.m. of seedbed.

The 2-gm. rate was ineffective. The 20-gm. rate gave only temporary control. The 30-gm. rate checked the spread of the disease, but the 50 and 100-gm. rates of copper sulphate excessively reduced seed germination and produced toxicity symptoms on seedlings.

Transplants from the beds treated with 30 and 50 gm. of copper sulphate developed normally, while those from the 100 gm. treatments remained stunted.

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THE YELLOW-DWARF VIRUS DISEASE OF CEREAL CROPS[1]

John W. Oswald[2] and Byron R. Houston[2, 3]

Yellow-dwarf, a new and destructive disease of cereals in California, was recently described in preliminary reports (13, 14). In the 1951 growing season a brilliant leaf yellowing of barley accompanied by moderate to severe stunting occurred in epiphytotic proportions in most barley producing counties in the state. Overall estimates indicate a 10 per cent loss in the 1951 California barley crop due to this disease, the situation being particularly acute in late-planted grain of which there was a large acreage in that year. Within a week after yellowing first appeared in barley in 1951, stunting and chlorosis of wheat and reddening of oats were observed. In many of the late-planted wheat fields losses in yields were extreme due to abundance of light-green stunted plants, many of which failed to head. Oat losses were equally severe. Occasional scattered barley and oat plants resembling this diseased condition have been noted since at least 1947, but no attempt has ever been made, with the exception of routine isolation, to ascertain the cause.

Comprehensive investigations initiated with the onset of the epiphytotic in April 1951 demonstrated that the disease was caused by a virus readily transmitted by at least 5 different species of grain-infesting aphids (13, 14, 15), but not by mechanical means, by seed, nor in the soil. Aphid inoculation studies indicated that the virus probably has a wide host range in the grass family (16). This knowledge should aid materially in understanding the epiphytology of cereal yellow-dwarf.

SYMPTOMS OF YELLOW-DWARF ON CEREAL CROPS.—*Barley.*—It was the characteristic golden yellowing of barley leaves together with stunting that prompted naming this new cereal virus disease yellow-dwarf. Symptoms on barley vary with the variety, but in all instances yellowing of leaves is the first visible evidence that a plant is infected.

The severity of symptoms is wholly dependent on plant age when infected. The following description refers principally to plants infected in the seedling stage. Within 12 to 15 days following inoculation leaves start to turn yellow usually at the tips (Fig. 1, B). The yellowing progresses downward, at first principally along the leaf margins, and gradually supplants all the normal green pigmentation of the leaves. The color is not the sickly chlorotic yellow of barley that develops under conditions of nitrogen deficiency, cold weather, or saturated soil, but rather is a brilliant golden yellow, sometimes almost orange. As pigment changes occur the last areas of the leaves to become yellow are those immediately adjacent to the main veins. In a few varieties, notably Atlas 46, yellowing may begin as irregular blotches midway in the leaf blade as well as at the tip (Fig. 1, C). These areas later coalesce and leaves become totally yellow. Areas of the plants that remain green are often a darker green than normal—almost a blue-green. Under conditions of high fertility this is particularly evident. Leaves on infected plants are erect and have the appearance of being thicker and stiffer than normal.

No mosaic mottle occurs in the symptom picture of yellow-dwarf other than the irregular blotches on the leaves that sometimes precede total yellowing. On some varieties a longitudinal green and yellow striping occurs on the new growth of infected plants (Fig. 1, E). The areas adjacent to and over the veins remain green whereas the interveinal tissues become chlorotic to yellow. A few varieties exhibit a marked serration of the leaf margins (Fig. 1, D).

Just as striking a symptom as leaf yellowing is the extreme stunting of barley by the yellow-dwarf virus. (Fig. 1, A). In the variety Blackhulless (C.I. 666), selected for its extreme susceptibility, infected plants are less than half normal size within a month following inoculation and rarely attain a height of over 6 in. (Fig. 1, F). The tillering process is actually stimulated, but elongation of internodes at the jointing stage in this variety is completely inhibited and no heads are produced. Growth ceases in such plants, but they remain alive for surprisingly long periods when not crowded out by adjacent healthy plants. In varieties such as Atlas 46 the stunting and yellowing are extreme in the early stages of growth, but as heading time approaches a partial recovery occurs with some of the late tillers yielding fairly normally. The root system of an infected plant is equally as retarded as the top growth. A drought that accompanied the outbreak of yellow-dwarf in 1951 exaggerated the damage, in that the shallow-rooted diseased plants were unable to reach or absorb sufficient water.

New growth produced by plants infected at intermediate growth stages (tillering to jointing) becomes typically yellow but stunting is less severe. Such plants manage to head but the grain produced is invariably less than normal and is of a lower test weight. When infection occurs at still later stages of growth the only visual evidence of yellow-dwarf in barley is a bright yellowing of the uppermost leaves, particularly the flag leaf.

Under natural conditions the disease often appears first in plants growing along the margins of fields. This is readily explainable in light of the discovery of aphid transmission and the susceptibility of grasses such as wild oats and barley that commonly grow along

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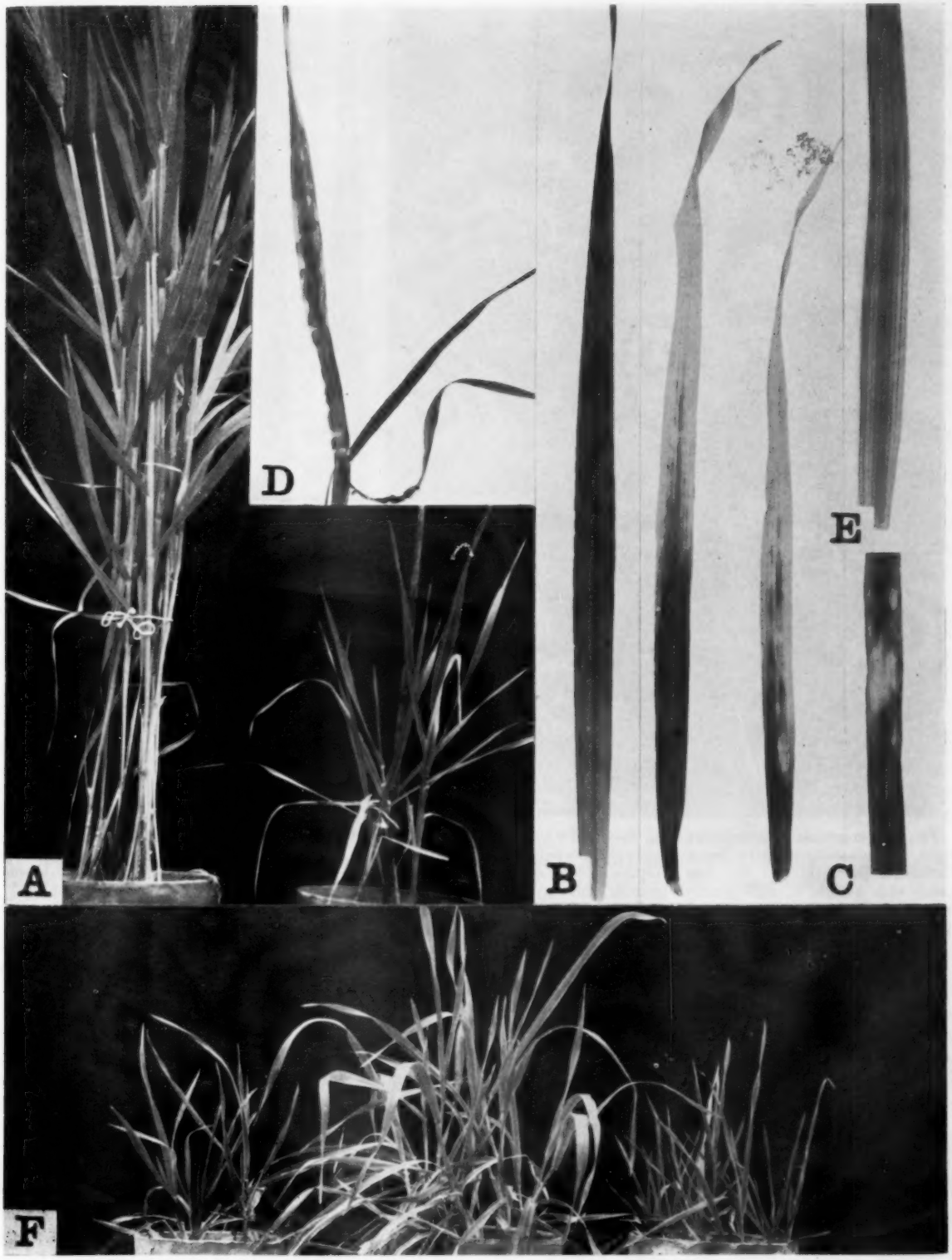

FIG. 1. Symptoms of yellow dwarf on barley. A) Club Marious—4 months old—left, healthy; right, plants of the same age inoculated at the seedling stage with aphids. B) Left, normal leaf; right, 2 leaves showing characteristic yellowing. C) Atlas 46 leaf exhibiting yellow blotches. D) Leaf serration symptom on C.I. 734. E) Longitudinal striping of young leaf. F) Blackhulless—2 months old—left, 5 plants inoculated with viruliferous corn aphids (*R. maidis*); on right with viruliferous English grain aphids (*M. granarium*); center, plants of same age on which non-viruliferous English grain aphids were fed.

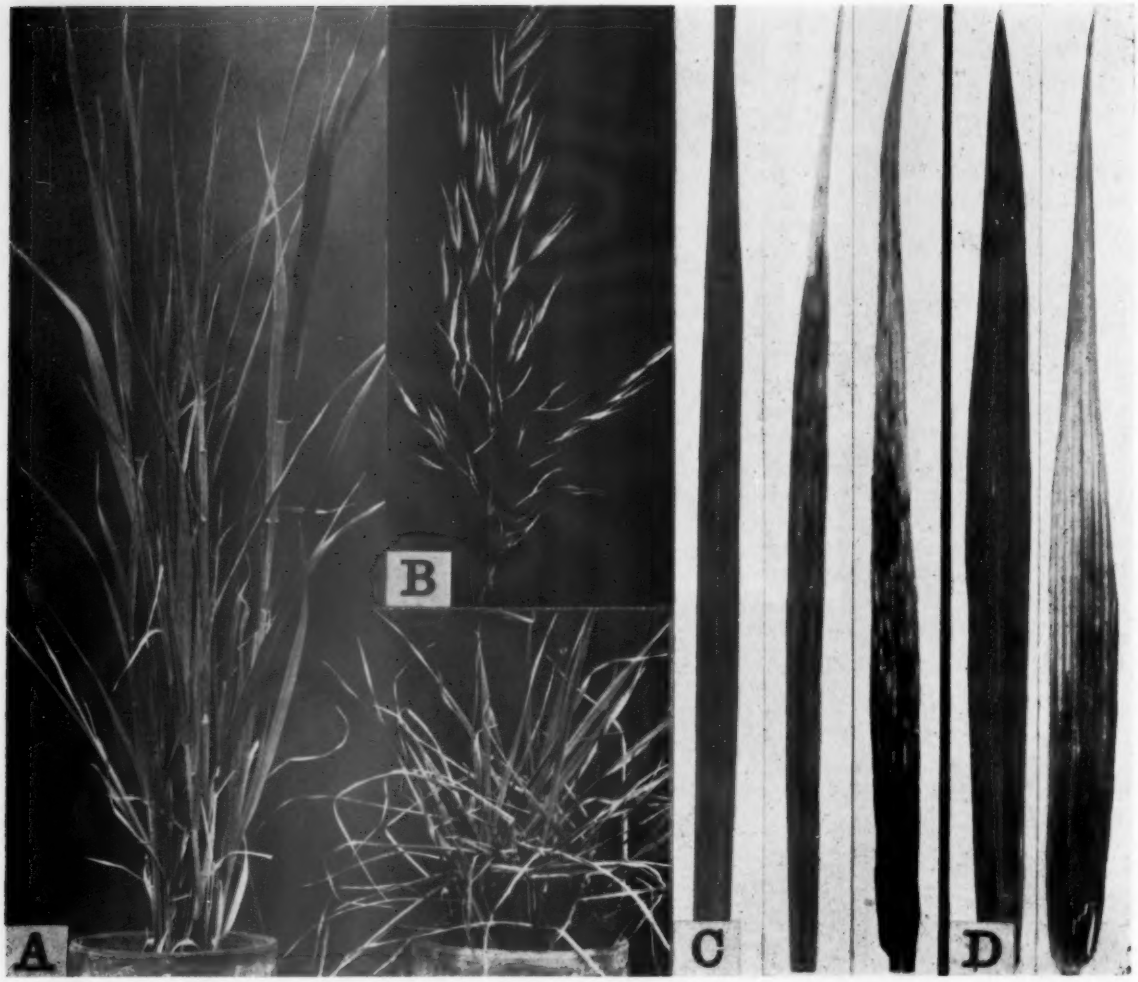

FIG. 2. Symptoms of yellow-dwarf on oats and wheat. A) Victoria oats, 4 months old, 5 plants per pot; left, healthy; right, inoculated with viruliferous apple-grain aphids (*R. prunifoliae*) at the seedling stage. B) Blasting of florets on California Red oats. C) Oat leaf symptoms; left, normal; center, yellow green-blotches; and right, small reddish spots, many with minute necrotic centers, that develop in advance of tip reddening. D) Wheat leaf symptom on a plant infected at a late stage of growth; left, healthy; right, leaf exhibiting tip yellowing with tendency for veins to remain green.

roadsides and ditchbanks. Occasionally, well-defined circular spots, 10–20 ft. in diameter, are encountered in which infection and damage is extreme. Rapidly multiplying colonies of aphids that had originally established themselves at the centers of such spots undoubtedly account for the spotted distribution of yellow-dwarf in nature.

The pattern of distribution of the disease in the most severely affected barley fields in 1951 gave the first clue to the nature of its cause. Without exception normal green plants could always be found scattered in such fields. Such healthy plants surrounded by stunted yellow plants were strong evidence that an infectious disease rather than some environmental factor was involved.

Oats.—Yellow-dwarf is just as destructive on oats. Symptoms and disease development in oats follow closely those described for barley. In oats, however, the most characteristic evidence of disease is red, rather than yellow, discoloration of the leaves.

The first sign of yellow-dwarf in most oat varieties occurs 2 to 3 weeks following inoculation in the form of yellowish-green blotches near the leaf tip not sharply delimited from normal tissues (Fig. 2, C). These blotches soon turn red to reddish-brown and coalesce, leaving the entire leaf tip red. The red pigmentation varies from yellow-red in such varieties as Victory and Palestine, through reddish-brown in Ventura and California Red to brilliant scarlet in Bond and Custis. At this stage the remainder of the plant has an over-all dark green to blue-green cast. The yellow islands in the green tissues develop in advance of the solid red discoloration as it progresses down the leaves. In a few varieties such as Traveler the disease begins as small reddish spots sometimes with necrotic centers (Fig. 2, C). Occasionally green streaks which are

associated with the main veins remain in the otherwise reddened tissues.

No true mosaic pattern develops but the longitudinal striping of younger leaves as described for barley is common in some oats (Fig. 1, E).

Under conditions of artificial inoculation, deep serration of the margins of newly emerging leaves is common in the most susceptible oat varieties. It is so extreme that tips of such leaves, and often as much as half the blade are completely severed before they emerge from the sheaths, resulting in plants with many half-leaves.

In oat plants inoculated in the seedling stage, the degree of stunting varies with the variety. In an extremely susceptible variety such as Victoria (Fig. 2, A) or Coast Black, infected plants are dwarfed by half within 5 weeks after inoculation and fail to head at all. An additional and consistent symptom of yellow-dwarf in oats is blasting of the flower parts (Fig. 2, B). This may involve from a few florets up to entire heads. Usually the basal florets are the most severely affected. Such blasted heads or portions of heads emerge from the boot in this condition, are completely devoid of seed, and are white as compared to the normal color of developing heads.

As with barley, the intensity of symptoms is directly correlated with the age of the plant when infected. Late infection of oats can be recognized only by the characteristic reddening of late emerging leaves.

Wheat.—Of the 3 major cereal crops, wheat is the most severely damaged by yellow-dwarf when it is infected in the seedling stage. The first indications of yellow-dwarf on seedling-inoculated plants are a darker than normal green color of the outer leaves, a chlorosis of the new growth, and an overall stunting. No characteristic pigment change develops other than a gradual chlorosis of the entire plant as growth stops. A suppression of tillering occurs in wheat. In the field as they approach maturity, seedling-infected plants are completely chlorotic and are ⅓ to ½ normal in size. Heading is sparse, if at all, and yield from such plants is practically negligible. Stunting is less severe on such plants grown in the greenhouse probably because moisture is continuously available to the small undeveloped root systems, a situation quite different from that in the field.

Infection of wheat with yellow-dwarf virus at later growth stages (after tillering) presents quite a different picture. In this case the first, and usually only, indication of the disease is a bright yellowing starting at the tips of newly formed leaves. Such leaves closely resemble those of infected barley except that there is more of a tendency in late-infected wheat for the veins in the yellow areas to remain green for a longer period (Fig. 2, D). There seems to be little if any stunting in wheat infected much after the tillering stage.

As with the other 2 cereals no mottle of a mosaic type occurs. Neither leaf serration nor head blasting have been encountered as symptoms of yellow-dwarf in wheat.

Rye.—On the basis of its behavior in the 1951 epiphytotic of yellow-dwarf, rye appears to be the most tolerant of the small grains to this disease. This observation, however, is limited to but 1 rye variety, Merced, which exhibited no discoloration and little if any stunting in fields where barley, oats and wheat were severely damaged. Artificial inoculations in the greenhouse have shown that rye can be infected and under such conditions a slight stunting occurs.

THE VIRUS NATURE OF CEREAL YELLOW-DWARF.—When, in mid-April of 1951, the barley in most areas of California suddenly turned yellow, practically all observers looked first to some environmental factor for the explanation. It was thought that the abnormal condition on all 3 cereal crops was the direct result of some set of peculiar environmental conditions, probably involving temperature, soil moisture, and soil fertility. As the season progressed and more information became available it appeared that environmental factors could not account for the disease. First of all, the disease was equally severe in areas with very diverse environmental conditions, both as to winter rainfall and temperature. Soil fertility could hardly be a factor when yellowing of barley invariably expressed itself most vividly in those fields with the heaviest nitrogen fertilization. Further evidence against a climatic factor being responsible was the fact that in all barley fields, no matter how badly affected, normal green plants were always scattered among the yellow, dwarfed plants.

Fungus root rots are important in certain of the older grain producing areas of California. The suddenness with which this disease struck and its wide distribution seemed to rule out root rot as a cause. This was verified by hundreds of isolations from the roots of badly diseased barley, wheat, and oat plants which yielded no consistent pathogenic fungi.

In 1951 aphid populations on grains, particularly barley, were higher than in many years. The possibility existed that the disease was due to direct aphid injury such as that caused by the green bug, *Toxoptera graminum* Rondani, in other areas (1). While this aphid is known to occur in southern California, no collections made in 1951 proved to contain the green bug. The fact that yellow-dwarf was severe in barley in areas where aphid populations were not extremely heavy was evidence that if aphids were involved they were more likely to be so in the role of vectors of a virus.

Failure to transmit the disease mechanically.—The first attmepts to prove that yellow-dwarf might be a virus disease were directed toward transmitting the virus by mechanical means. In 4 different experiments juice extracted from diseased plants was rubbed onto seedlings in the 1–3 leaf stage with carborundum powder used as an abrasive. In these trials infected barley, wheat and oats were each used as source plants and seedlings of the most susceptible varieties of each of these cereals were inoculated. From a total of 284 plants inoculated only 1 developed yellow [illegible]. In

view of the subsequent proof that the disease is caused by a virus readily transmissible by aphids, this 1 plant is looked upon as an accidental aphid transmission in the greenhouse.

Numerous later attempts to transmit yellow-dwarf mechanically from aphid-infected greenhouse plants were all unsuccessful. The results indicate that yellow-dwarf cannot be transmitted mechanically by ordinary means of rubbing seedlings with juice extracted from diseased plants.

Aphid transmission of the yellow-dwarf virus.—During the 1951 epiphytotic of yellow-dwarf 4 different species of grain-infesting aphids: corn aphid, *Rhopalosiphum (Aphis) maidis* (Fitch); apple-grain aphid, *Rhopalosiphum prunifoliae* (Fitch); grain aphid, *Macrosiphum granarium* (Kirby); grass aphid, *Macrosiphum dirhodum* (Walker), were collected.[4] Colonies of each were established by caging aphids on diseased barley, wheat and oat plants that had been transplanted from the field into the greenhouse. For the transmission studies seedling plants were grown in 6-in. pots and at the 2-leaf stage were thinned to 5 per pot. Ten aphids that had been reared on diseased plants were transferred to each seedling by means of a camels-hair brush. The pots were then covered by muslin cages for a 3-day period after which the aphids were removed by a 1 per cent parathion spray. In the initial phases of the studies before non-viruliferous aphid colonies were available, pots caged in the same manner but with no aphids were used as controls.

Varieties of the 3 cereals that had proved to be extremely susceptible under field conditions were selected for test plants. These included Blackhulless (C.I. 666), C.I. 734 and C.I. 998 barley varieties, Ventura and Coast Black oats and Baart 38 wheat.

All aphid colonies in the first trials were derived from aphids collected directly from the field. For positive proof of aphid transmission of a virus and to eliminate completely the possibility of direct aphid injury being a factor, it was necessary to begin with nonviruliferous colonies. These were obtained by placing winged aphids in small glass vials containing moistened filter paper and capped with cheese cloth. After 24 hours the nymphs which had been produced in the vials were transferred to healthy barley seedlings that had been caged since emergence. Colonies of the 4 aphid species produced in this manner all proved to be non-viruliferous and in all subsequent transmission tests were used as checks against direct aphid injury being the cause of the symptoms.

Pots of inoculated plants were maintained in the greenhouse at temperatures of 24–28°C. and within 2–3 weeks could be examined for the development of the disease. Distilled water was used exclusively on the plants to avoid the tip burning and leaf spotting due to excess boron that develops on small grains when continually watered from the tap source available.

The data (Table 1) demonstrate conclusively that aphids of each species, when fed on diseased barley plants and then transferred to barley, wheat and oat seedlings, induced yellow-dwarf to develop. The average percentage of transmission to the three cereals ranged from 93.2 per cent for the grain aphid (*M. granarium*) to 26.8 per cent for the grass aphid (*M. dirhodum*).

The disease that developed on the inoculated plants of all 3 crops was in all respects identical to that occurring naturally in the field in 1951. Within 2–3 weeks after the 3-day aphid feeding period, symptoms appeared and progressed in intensity as the plants became older. The fact that the disease developed after such a short feeding period and became more intense with time was strong evidence that a virus rather than direct aphid injury was involved. To be certain of this fact, non-infective colonies of the 4 aphids obtained as described above were utilized in all subsequent work.

Non-viruliferous aphids of the 4 species were placed upon diseased plants and allowed to feed for a week. Ten aphids from such colonies were caged on each barley seedling (Blackhulless) for a 3-day period. A parallel series of tests with aphids that had never fed on diseased plants (non-viruliferous) was carried out at the same time. The results (Table 1) substantiate the fact that yellow-dwarf is caused by an aphid-borne virus, and show further that no yellow-dwarf developed when non-viruliferous aphids were used. None of these 4 aphid species in the 3-day feeding period caused any systemic effect on the barley seedlings.

Transmission tests with a fifth aphid species, *Toxoptera graminum* (greenbug) are included in Table 1 (15). Since the greenbug is of such extreme importance and occurs so widely in many of the major cereal producing regions, it was considered important to determine whether or not this aphid could act as a vector of the yellow-dwarf virus. It is known to cause severe injury to the 3 major cereal crops (1) but in only 1 instance has it been reported as a vector of a virus—that of western wheat mosaic (2). As mentioned above this aphid was not encountered in the 1951 epiphytotic of yellow-dwarf in California, but on numerous occasions it has been reported in the State, particularly from the southern part in the spring.[5] The results in Table 1 show that the greenbug can act as a vector of the yellow-dwarf virus. The 37 per cent transmission obtained is somewhat higher than that found for the grass aphid, but considerably lower than that obtained with the other 3 proven vectors.

It should be pointed out that in the 3-day feeding period on barley seedlings both the infective and non-infective greenbugs caused direct feeding injury in

[4] The authors are indebted to Dr. E. O. Essig of the Department of Entomology, University of California, Berkeley, California for identification of the aphid species.

[5] Since no colony could be located in California at the time of these tests, a culture of greenbugs was kindly furnished to the authors by the Division of Entomology at Kansas State College.

TABLE 1. *Transmission of the cereal yellow-dwarf virus to barley, wheat and oat seedlings by 5 species of grain-infesting aphids with colonies collected directly from the field and with aphids originating from non-viruliferous colonies*

Aphid Species	Aphid colonies from field collections[a]						Aphids originating from non-viruliferous colonies			
							viruliferous aphids[b]		non-viruliferous aphids[c]	
	Barley		Wheat		Oats		Barley		Barley	
	no. inoc.	% infected	no. inoc.	% infected	no. inoc.	% infected	no. plants	% infected	no. plants	% infected
Rhopalosiphum prunifoliae	184	82.6	20	100.0	30	96.7	40	100.0	15	0.0
Rhopalosiphum maidis	75	64.0	9	88.9	30	50.0	20	100.0	10	0.0
Macrosiphum granarium	310	91.0	10	100.0	125	98.4	40	80.0	15	0.0
Macrosiphum dirhodum	50	36.0	18	0.0	3	33.3	40	34.9	10	0.0
Toxoptera graminum[d]	–	–	–	–	–	–	151	37.1	40	0.0

[a] Aphid colonies originating directly from field collections were first reared on diseased barley plants and then approximately 10 aphids were placed on each seedling and left for 3 days.
[b] Non-viruliferous aphids were placed on diseased barley plants and allowed to feed a week before transferring 10 to each barley seedling for the 3-day period.
[c] Non-viruliferous aphids were transferred directly to each barley seedling without any feeding on diseased plants.
[d] This aphid (greenbug) was not encountered in the field in 1951.

the form of localized yellow spots with necrotic centers. In this short feeding period, however, no permanent damage was done to the plants by greenbugs. There was never any difficulty in distinguishing greenbug feeding damage from the characteristic symptoms of the yellow-dwarf virus.

The yellow-dwarf virus has been transferred from diseased barley, wheat and oats to seedlings of each of these cereals. Symptoms of the disease in a given variety are identical regardless of the source of the virus.

Failure to transmit the yellow-dwarf virus through seed or soil.—Seed collected from naturally diseased barley plants in May 1951 was grown in greenhouse flats to check seed transmission of the yellow-dwarf virus. Of 1110 seeds planted, 951 produced plants (85.7 per cent germination). The seed from such severely diseased plants was light in weight and, as one would expect, yielded weaker plants than those from plump kernels. None of the 951 plants, however, showed any symptoms whatsoever of yellow-dwarf.

To determine whether or not the yellow-dwarf virus is soil-borne, as are some of the cereal viruses (4, 5, 7), soil from severely diseased spots in the field was brought into the greenhouse. Some of the most susceptible varieties of wheat, barley and oats were grown to maturity in flats of this soil. In no instance did any plant develop any indication of yellow-dwarf.

PERSISTENCE OF THE YELLOW-DWARF VIRUS IN THE APHID.—In being aphid transmissible but not mechanically transmissible, the cereal yellow-dwarf virus resembles such viruses as potato leaf roll, sugar beet yellows and sugar beet yellow-net. These have all been shown to be persistent viruses (19, 21, 23) as defined by Watson and Roberts (24).

To determine whether or not yellow-dwarf is persistent in the insect vector, the English grain aphid, *M. granarium*, was used. Fifteen infective winged or large apterae were placed on each of 18 Blackhulless barley seedlings. After a 24-hour feeding period the surviving apterae on each seedling were transferred to 18 new seedlings. This was repeated at 24-hour intervals through 5 serial transfers. Only 5 seedlings were available for the sixth transfer.

Of 18 seedlings inoculated at each 0, 24, 48, 72 and 96 hours after removal of the aphids from the virus source, 18, 17, 16, 15 and 12 plants respectively developed yellow-dwarf. Four out of 5 seedlings inoculated with aphids in the sixth serial transfer (120 hours after removal from virus source) became diseased. These results clearly indicate that the yellow-dwarf virus can be considered a persistent virus.

The fact that the virus is persistent, probably for the life of the insect, helps to explain the rapid build-up of the disease under field conditions. A single winged, infective aphid might well infect a great number of plants.

VARIETAL REACTION IN CEREAL CROPS TO YELLOW-DWARF.—As pointed out in the description of symptoms of cereal yellow-dwarf, there is considerable variation among the different varieties in their reaction to the disease.

Barley and Oats.—A number of barley and oat varieties, including all those commonly grown in California and those selected under field conditions for either extreme tolerance or susceptibility, were tested by aphid inoculation at the seedling stage in the greenhouse. On the basis of these trials the varieties were placed into 4 different reaction groups.

Those barleys (C.I.'s 1227, 1237, 2376, Abate) rated

as highly resistant exhibit no stunting and little if any leaf yellowing. These 4 varieties were selected as resistant by the authors and Dr. C. W. Schaller of the Department of Agronomy during the 1951 epiphytotic. None are of commercial value, but all represent possible parental material for a disease resistance breeding program. No oat varieties with this degree of resistance to yellow-dwarf have been encountered.

Into the group ranked as tolerant fall the commercial varieties, Rojo (C.I. 5401) and Hannchen (C.I. 531) of the barleys and Kanota (C.I. 839) and Bond (C.I. 2733) of the oats. Such varieties are stunted only slightly but show considerable pigment changes in the leaves. Varieties in this class manage to head out and yield somewhat normally despite seedling infection with yellow-dwarf. The experimental inoculations in the greenhouse substantiate the field observations of 1951 which rated Rojo best of the barleys and Kanota outstanding among the oats wherever yellow-dwarf was a factor. Of the Eastern oats tested, Bond was the only variety falling into this class.

Those oat and barley varieties rated in the third or intermediate class are stunted at maturity by approximately half. Symptom expression is severe. They normally produce heads but yields are drastically reduced. A few varieties in this group, such as Atlas 46 (C.I. 7323), are severely stunted and yellow in the early stages of growth but as heading occurs there is considerable recovery. California Mariout (C.I. 1455), Arivat (C.I. 6573) and Vaughn (C.I. 1367) are the other California barley varieties in this group. Of the California oats, Westdale (C.I. 2747), Ventura (C.I. 3989) and Palestine (C.I. 2696) exhibit this intermediate reaction. Other oats reacting similarly are Custis (C.I. 2041), Lee (C.I. 2042), Richland (C.I. 787) and Victory (C.I. 1197).

Two varieties of oats, California Red (C.I. 1026) and Coast Black, and of barley, Tennessee Winter (C.I. 4633) and Club Mariout (C.I. 261) grown commercially in California rank in the extremely susceptible class. When infected in the seedling stage these are so severely dwarfed (often by three-quarters) that they rarely if ever produce heads. Four other barleys, Blackhulless (C.I. 666), C.I. 734, C.I. 998 and C.I. 999, were observed in the field to be highly susceptible and inoculations proved this to be true for all 4. Three eastern oat varieties, Letoria (C.I. 3392), Traveler (C.I. 4206) and Victoria (C.I. 2401) fall into this category.

It should be pointed out that in the greenhouse where conditions are favorable in all respects for plant growth the degree of stunting due to yellow-dwarf is actually less than that encountered under field conditions.

Wheat.—Although no experimental data are available to measure the relative susceptibility of commercially grown California wheats, field observations indicate that the 3 most common varieties, Baart 46, White Federation 38 and Ramona 50 are highly susceptible. Sonora 37 appears to be the most tolerant of the California wheats.

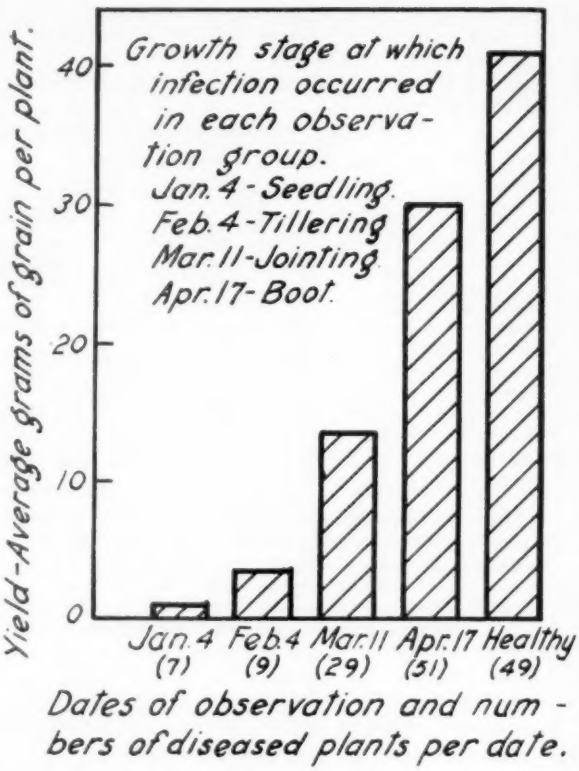

FIG. 3. Correlation between growth stage of the plant when infected with yellow-dwarf and ultimate yield of grain of the barley variety, Atlas 46 planted November 1951 and harvested June 1952.

EFFECT OF YELLOW-DWARF ON YIELD OF CEREAL CROPS.—Any attempts to measure the effect of yellow-dwarf on cereal yields must take into consideration the growth stage of the plants when infection occurs.

Barley.—In an experimental planting of barley made in November 1951 by Dr. C. W. Schaller of the Department of Agronomy randomized rod rows of the 3 principal California varieties, Atlas 46, California Mariout and Club Mariout were included. Yellow-dwarf was uniformly distributed in this plot by January to the extent of 3–4 per cent and by February the incidence had increased to 15 per cent. With the advent of warmer weather, which brought about a build-up of aphids, the incidence of disease rose to 23 per cent by mid-March and to 50 per cent by April 17.

At monthly intervals starting in January, 6 rows of each variety were examined and every plant showing yellow-dwarf symptoms was staked to indicate the date the disease was first discernible. On April 17, the last observation date, all varieties were fully headed and in the soft dough stage. No attempts were made to read the disease beyond this date, and for the purposes of this experiment all plants that showed no symptoms on April 17 were considered healthy. Plants of each variety were grouped at harvest according to the date the disease was first observed. These composite samples

were then threshed and the average weight in grams of grain per plant was calculated for each variety for each observation date.

There was a direct correlation between time of infection and ultimate yield of barley (Fig. 3). The incubation period of the virus is 2–3 weeks and therefore, actual infections predate each date of observation. Those plants showing disease on January 4 presumably were infected in the seedling stage about December 15; those on February 4 in the early tillering stage; those on March 11 in the jointing stage; and those on April 17 in the boot to early heading stage. Such predating of actual infection is of course only approximate. There is the possibility that incubation period may have been lengthened somewhat by the cool weather prevailing in December and January and further, that incubation period might be longer than 2–3 weeks for plants infected at late stages of growth.

Grain yields of all 3 varieties when infected in the seedling stage were reduced over 95 per cent. Yields of Atlas 46 and California Mariout, when infected at jointing, were cut by approximately 70 per cent and that of the more susceptible Club Mariout over 90 per cent. With late infection in the heading stage all showed approximately the same order of reduction—15 to 25 per cent.

In measuring yield under the conditions of a natural field spread such as this, there are the problems of healthy plants adjacent to diseased ones yielding higher than normal and at the same time diseased plants adjacent to healthy ones being crowded out and yielding less than their potential. That is to say, if no disease had occurred, the healthy yields would have been lower or, conversely, if every plant had been infected at the same growth stage, diseased plant yield would have been higher. Thus, yields in this experiment calculated on an acreage basis would be misleading, but the important thing here is the relative yields of plants infected under conditions of natural field spread.

Wheat.—To gain some knowledge of the effect of seedling infection on wheat yields, 24 plants of each of 3 varieties, Baart 38, White Federation 38 and Ramona 50 were inoculated in the greenhouse and transplanted to the field. A comparable number of noninoculated checks were put out at the same time. Yields in gm. of grain per plant were as follows: Baart 38, 6.6 gm. per healthy plant, 0.8 gm. per diseased plant; White Federation 38, 9.0 gm. as against 0.7 gm; and Ramona 50, 6.1 gm. as against 1.2 gm.

Oats.—No experimental yield data are available for this crop, but the picture is the same as for barley and wheat—severe reduction with seedling infection and less damage in the corresponding later infection dates.

Discussion.—Every indication points to the fact that cereal yellow-dwarf is quite different from all previously described cereal virus diseases. It differs from the eastern soil-borne wheat mosaic, from plains wheat mosaic, and from wheat streak mosaic (western wheat mosaic) diseases described by McKinney (4, 5, 6, 7, 8) in 3 major respects: 1) the yellow-dwarf virus is aphid transmissible; 2) it cannot be transferred by mechanical means; and 3) the symptoms more nearly resemble those of a yellows type virus disease than the mosaic type. Its failure to persist in soil further distinguishes the yellow-dwarf virus from eastern wheat mosaic (wheat mosaic rosette) and the oat apical mosaic and eyespot viruses (4, 7). The 2 cereal mosaic diseases described in Russia (20, 25) are leafhopper rather than aphid-transmitted.

Viruses affecting barley have been mentioned previously only in connection with wheat mosaic diseases (3, 4, 5, 9, 20, 22, 26) and false stripe (11). The symptoms on barley affected by false stripe as described by McKinney, plus the fact that this disease is readily seed- and mechanically-transmissible, clearly distinguish it from yellow-dwarf.

Of the oat diseases, yellow-dwarf closely resembles red-spot mosaic described by Rosen (17). Recently Rosen (18) has described what he considers to be yellow-dwarf in Arkansas and differentiates it from red-spot mosaic; however no aphid transmission trials were presented to confirm this. Yellow-dwarf also resembles some of the oat troubles referred to as red leaf. As pointed out by McKinney (10) there are many complex factors that may induce pigment disorders in small grains other than infectious diseases. Yellow-dwarf, however, does cause a red pigmentation and other symptoms on oats so similar to the so-called red leaf disease that only aphid recovery studies can positively determine whether or not the yellow-dwarf virus is a factor.

In 1952 Moore (12) described a disease or diseases of oats he called blue dwarf and red leaf which are caused by a virus that apparently is very similar if not identical to the cereal yellow-dwarf virus. Both blue-green stunting and leaf reddening are characteristic symptoms of yellow-dwarf on oats. On 3 other points blue dwarf and red leaf are similar to cereal yellow-dwarf: 1) neither disease can be transferred mechanically; 2) both are aphid transmissible (*R. prunifoliae* and *M. granarium*); and 3) incubation periods of both viruses are the same.

Because most of the aphid vectors of yellow-dwarf, particularly the green bug, occur commonly in a majority of the cereal producing areas of this country, a constant vigil should be kept on this new and potentially important cereal virus disease.

Yellow-dwarf unquestionably has been present at a low ebb in California cereal crops for some time. All workers involved with small grains at the California Experiment Station agree that diseased barley and oat plants resembling yellow-dwarf have been observed during the last few years. Current studies showing that the virus has a wide host range in the grass family will aid materially in explaining the 1951 epiphytotic.

SUMMARY

Yellow-dwarf, a new and damaging disease of cereals, occurred in epiphytotic proportions in California

in 1951. The severity of symptoms and ultimate effect on yield of barley, wheat and oats is dependent on the age of the plants when infected. Characteristic of yellow-dwarf on barley is a golden yellowing of the leaves accompanied by moderate to severe stunting. On infected oats, stunting, leaf reddening and head blasting occur. Wheat infected in the seedling stage is chlorotic and severely dwarfed. Infection at later growth stages induces a yellowing of the upper leaves of barley and wheat and a corresponding reddening of oats.

Yellow-dwarf is caused by a virus that could not be transferred by mechanical means but which is readily aphid transmissible. All five grain-infesting aphids tested, *Macrosiphum granarium*, *M. dirhodum*, *Rhopalosiphum maidis*, *R. prunifoliae*, and *Toxoptera graminum* have proven to be vectors. Tests with non-viruliferous aphids of all 5 species eliminate the possibility of direct aphid injury being the cause of yellow-dwarf. The virus is persistent up to 120 hours in the aphid vectors.

All attempts to transmit yellow-dwarf through seed and soil were negative.

Four barley varieties, C.I. 1227, C.I. 1237, C.I. 2376 and Abate were found to be highly resistant. Rojo and Hannchen barleys and Kanota and Bond oats are considered tolerant. The barley varieties, Club Mariout, Tennesse Winter and Blackhulless and the oats, Victoria and California Red, rate as extremely susceptible.

Studies on barley showed a direct correlation between the time of infection and effect of yellow-dwarf upon yield of grain. Yield was reduced by 95 per cent in 3 barley varieties naturally infected at the seedling stage in the field.

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INDUCING DISEASE RESISTANCE WITH PLANT GROWTH-REGULATORS [1]

David Davis [2] and A. E. Dimond

In a recent publication Davis and Dimond (9) noted a direct correlation between the chemotherapeutic activity of 2 synthetic organic chemicals and their capacity to alter the host's metabolism. Changes in gross morphology and concentration of certain biochemical constituents of the host were described. Furthermore, a fungitoxic moiety was not detected in the tissue of treated plants. Accordingly, we hypothesized that these compounds increased resistance to disease by inducing changes in the host's metabolic processes unfavorable for disease development.

Many plant growth-regulators are known to influence profoundly the biochemical processes of higher plants (3, 5, 25, 15) and are relatively innoxious to certain fungi (7, 12, 16, 17, 19). Their capacity to induce changes in disease susceptibility could be used as further evidence in support of the hypothesis of changed disease susceptibility through altered metabolism. Consequently, a group of chemically divergent types of plant growth-regulators with potentially varied activity on plant metabolism were chosen for investigation.

Experimental.—With the exception of the method of application of the chemicals, the chemotherapy assays were performed on the Fusarium wilt disease of tomato as prescribed by Dimond et al (10). Randomized blocks containing 5 plants per treatment were used in each chemotherapy assay. Three or more assays per treatment were performed in which inoculation followed treatment and one in which inoculation preceded treatment. The plant growth-regulators were applied to the foliage rather than to the sand culture on which the tomatoes were grown. To prevent chemical contamination of the tomato sand cultures, the tomato pots were covered with a notched piece of fiber board, inverted, with the foliage dipped into a solution of plant growth-regulator for 15 seconds. The plants remained inverted until thoroughly dry. In no instance did a reapplication of the plant growth-regulator follow by less than 48 hours. *In vitro* fungitoxicity of the plant growth-regulator was measured as previously described by Davis and Dimond (9). However, Richard's nutrient was replaced by Czapek's nutrient. The fungitoxicity assay was performed twice. Sugar analysis of the tomato plant tissue was performed on a set of plants grown and treated concurrently and identically with those used in the chemotherapy assay. One set of treated plants was harvested 10 days after the initial treatment (another set was inoculated on the same day for the chemotehrapy assay) and macerated in a Waring blendor. A hot water extract (80° C.) of the macerated tissue was used for the sugar analysis. The analysis was performed by AOAC methods (2).

Results.—Most of the plant growth-regulators tested reduced the Fusarium wilt disease of tomato. Thus 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), 2,3,5-triiodobenzoic acid (TIBA), β-naphthoxyacetic acid (NOA), and indole-3-acetic acid (IAA) reduced disease to 0, 0, 40.4, 54.7 and 67.2 per cent of that in the untreated check plants (Table 1). The reductions in disease induced by 2,4-D, NAA, TIBA or IAA in a series of experiments are significant. The chemotherapeutic activity of NOA although not statistically significant followed the same pattern exhibited by the effective plant growth-regulators. Coumarin, phenylacetic acid and dl-mandelic acid did not display chemotherapeutic activity at 500 p.p.m. The chemotherapeutic potency of the plant growth-regulators is related to the interval between time of inoculation and treatment (Table 1). The longer the time that inoculation follows treatment the greater the potency of the plant growth-regulators. This effect is best characterized by the action of 2,4-D. When inoculation preceded the treatment by 4 days disease susceptibility was not appreciably affected. In plants inoculated 4 days after treatment with 2,4-D disease was reduced to 33 per cent of the check. Disease symptoms were absent in plants inoculated 10 days after treatment with 2,4-D (Table 1). The effectiveness of treatment in relation to time of inoculation is significant for 2,4-D and NAA. Although a similar trend was observed with TIBA, NOA and IAA the results are not statistically significant.

There is a wide disparity between chemotherapeutic activity and fungitoxicity of plant growth regulators. This is well demonstrated by a direct comparison of the concentrations applied to the fungus in artificial culture (Table 2) and to the host (Tables 1 and 3).

When calculated on the basis of the maximum concentration that theoretically could have entered the host, the disparity is further increased. Thus, 10 ml. (the amount that adhered to the foliage) of a 5 p.p.m. solution of 2,4-D applied to the tomato foliage must have been diluted to at least 1 p.p.m. in the whole plant (the treated tomato plants weighed 50.6 gm. The specific gravity of the tomato plants was less than 1.0). The same calculated proportional reduction in concentration is applicable for all the other plant growth-regulators. One need only multiply by the total number of applications per treatment in Tables 1 or 3 to determine the theoretical maximum concentration of any given plant growth-regulator in the treated host. Even on the assumption that 2,4-D was

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[2] Plant Pathologist, Crop Protection Institute.

TABLE 1.—*The chemotherapeutic activity of plant growth-regulators*

Compound	Conc. p.p.m.	No. appl.	Initial treatment in relation to time of inoculation: 4 days after inoculation — Vascular discoloration % Check	4 days before inoculation — Vascular discoloration % Check	10 days before inoculation[a] — Vascular discoloration % Check
2,4-Dichlorophenoxyacetic acid	5	1	78.0	33.0	0.00
α-Naphthaleneacetic acid	50	1	115.0	12.0	0.00
2,3,5-Triiodobenzoic acid	10	2	65.0	55.3	40.4
β-Naphthoxyacetic acid	60	2	84.5	40.9	54.6
Indole-3-acetic acid	500	2	92.2	77.0	67.2
Inoculated Check	—	—	100.0	100.0	100.0

[a] See Table 3 for concentrations of plant growth-regulators applied and number of applications.

restricted to the host's vessel elements and tracheids, the *Fusarium oxysporum* f. *lycopersici* present in the xylem was not exposed to inhibitory concentrations of 2,4-D. The total volume of vessel elements and tracheids in tomato as interpolated from Artschwager's (1) anatomical descriptions of the potato plant is at least 5 per cent of the total plant volume. Accordingly, the xylem of the treated tomato plant could have contained no more than 20 p.p.m. 2,4-D. Likewise plants treated with NAA could not have contained inhibitory concentrations commensurate with its disease reducing capacity. Although TIBA, NOA, and IAA could have theoretically been present in the xylem at inhibitory concentrations by the above method of calculation, it is highly unlikely that these compounds are restricted to the xylem and furthermore some of these materials are apparently inactivated or fixed by the plant after treatment. The normal growth of the tissues, derived from meristems developed some time after treatment, is evidence that plant growth-regulators are inactivated in the plant. The breakdown of IAA by the enzyme indoleacetic acid oxidase has been demonstrated (22).

TABLE 2.—*In vitro fungitoxicity of various plant growth-regulators to F. oxysporum f. lycopersici*

Compound	Conc. p.p.m.	Mycelial weight (mgm.)	% Check
2,4-Dichlorophenoxyacetic acid	1000	.0261	43
	500	.0424	70
	100	.0606	101
α-Naphthaleneacetic acid	1000	.0139	23
	500	.0182	30
	100	.0607	101
2,3,5-Triiodobenzoic acid	1000	.0097	16
	500	.0274	45
	100	.0333	55
β-Naphthoxyacetic acid	1000	.0170	28
	500	.0293	49
	100	.0494	82
Indole-3-acetic acid	1000	.0388	64
	500	.0557	92
	100	.0743	123
Check	.....	.0603	100

The chemotherapeutically active plant growth-regulators consistently altered the metabolism of the host. Symptomatic of a modified metabolism are the formative effects on the plant and the reductions in plant weight and reducing sugar content of tomatoes treated with the plant growth-regulators (Table 3). However, the order in which disease susceptibility is decreased by the plant growth-regulators is not directly correlated with the order of decrease in plant weight or reducing sugar content. Furthermore, the reduction in plant weight is not consistently related to the decrease in reducing sugar content.

Root damage as described by Keyworth and Dimond (14) was apparently not a factor influencing chemotherapeutic activity. First, the roots were not visibly injured. Second, although all of the chemotherapeutically treated plants showed a reduction in root weight, the reductions were not directly related to the chemotherapeutic potency of the plant growth-regulators. IAA, one of the less potent chemotherapeutants, caused the greatest reductions in root weight. 2,4-D inappreciably affected the root weight but completely eliminated disease symptoms (Table 3). This does not eliminate the possibility that the plant growth-regulators induced changes in the morphology of the roots (or other parts of the plant) unfavorable for disease development. Nevertheless, any morphological changes induced by the plant growth-regulators are presumably due to changes in the host's metabolism.

DISCUSSION.—Two criteria in substantiation of the hypothesis of changed disease susceptibility through altered metabolism presented by the authors (9) have been fulfilled. Thus, compounds with a) known capacity to alter the host's metabolism and b) poor fungitoxic properties have been shown to alter susceptibility to disease. Of particular significance is the increase in chemotherapeutic activity of the plant growth-regulators the longer the time of inoculation follows treatment. Since the evidence indicates that a large part of the plant growth-regulator is adsorbed within the first few hours after treatment (18, 24) it would appear more likely that if the plant growth-regulator were acting as a *direct* fungitoxicant or toxin inactivator its

TABLE 3.—*The relation between chemotherapeutic activity, plant weight, and reducing sugar content of plants treated with plant growth-regulators*

Compound	Conc. p.p.m.	No. of applications	Disease analysis: Vascular discoloration % Check	Plant Weight Analysis: Total plant fresh weight % Check	Plant Weight Analysis: Root fresh weight % Check	Sugar Analysis: Reducing sugars before inversion % Check
2,4-Dichlorophenoxyacetic acid	5	1	0.00	78.5	86.0	59.3
α-Naphthaleneacetic acid	200	1	0.00	55.0	66.2	15.1
2,3,5-Triiodobenzoic acid	10	1	40.4	69.5	73.2	71.7
β-Naphthoxyacetic acid	30	2	54.6	71.5	80.0	53.8
Indole-3-acetic acid	500	2	67.2	63.0	52.2	66.6
Untreated check[a]	....	....	100.0	100.0	100.0	100.0

[a] The untreated check plants weighed 64.3 gm., the roots weighed 7.1 gm. and the tissues contained 3.86 mgm. (expressed as Cu_2O equivalents) of reducing sugars before inversion per gram of fresh weight.

effect should be more pronounced the shorter the interval between inoculation and treatment. The change in biochemical constituents of plants treated with plant growth-regulators do, however, follow a time pattern, which can be correlated with changes in disease susceptibility. Carbohydrate, nitrogen, vitamin and mineral content of plants treated with growth-regulators have been shown to change progressively over a 6-day period (3, 25, 15, 5). The inoculation-treatment sequence experiment in this study serves to substantiate the concept that synthetic organic chemicals capable of modifying the host's metabolism may alter its susceptibility to disease.

Although TIBA and IAA are physiological antagonists (11, 23), both increased disease resistance (Table 1). This phenomenon can be reconciled with the findings of Thimann and Bonner (23) that TIBA, a hemi-auxin, enhances IAA activity when the concentration ratio of TIBA to IAA is small but inhibits IAA action when the concentration ratio is large. These authors hypothesized that TIBA and IAA are competing for the same active centers in the cell. The chemotherapeutic activity of TIBA may then be explained by the enhancement of residual IAA in the host tissues. In preliminary tests we have noted that TIBA in combination with IAA produces an additive chemotherapeutic effect. However, the additive effect tends to diminish with increasing concentrations of IAA. Thus, (irrespective of the validity of the Thimann and Bonner hypothesis) by altering the balance of 2 compounds which influence the metabolism of the host, the chemotherapeutic response each induces independently is partially counteracted. This is further evidence that IAA and TIBA are not reducing disease by direct fungitoxic action.

The chemotherapeutic activity of a group of phenoxy compounds has been attributed by Crowdy and Wain (8) to their inherent fungitoxic properties. These compounds are, however, innoxious to the test fungus at concentrations of 100–500 p.p.m. The recent findings of Burstrom (6) that α-(p-chlorphenoxy) isobutyric acid antagonizes IAA in higher plants may necessitate a reinterpretation of the mode of action of the chemotherapeutants studied by Crowdy and Wain (8).

The question arises as to how a modified host metabolism might influence disease resistance. Conceivably, by a) regulating the growth of the parasite and/or the elaboration of the toxin(s) or b) modifying the response of the host to the pathogen and/or the deleterious products of the host-parasite interaction. Which of these factors is involved in our studies is uncertain. However, we have been unable to dissociate disease symptoms in the stem with presence of the parasite. Accordingly, it would appear more likely that the plant growth-regulators reduce disease by inducing changes in host metabolism which regulate the growth of the parasite and/or the elaboration of toxins.

The capacity of the plant growth-regulators to induce resistance in 1 host-parasite relation does not necessitate that it act similarly in others. Rowell (20) has already demonstrated that 2,4-D increases the susceptibility of tomato to early blight. Furthermore, the modifying influence of light (4), temperature (13) and nutrition (21) on plant growth-regulator activity could cause the plant growth-regulator to induce modified chemotherapeutic responses under appropriate environmental conditions.

The future of these compounds as practical chemotherapeutants will be decided by a number of factors. The most obvious is whether possible deleterious effects of the compound are more or less important than losses caused by certain diseases which at present cannot otherwise be controlled economically. The relative deleterious and beneficial effects will be governed by the nature of the compounds, disease, environment, dosage and method of application. That certain of these factors are important in influencing the effect of plant growth-regulators, *e.g.*, on fruit set and drop, is well known. Further experimentation is, therefore, necessary before any concrete statements are made on the practicality of plant growth-regulators as chemotherapeutants. A most fruitful approach in applying the thesis presented in this paper may lie in the study of structurally modified plant growth-regulators. Proper modifications may result in compounds with little

deleterious effect, and yet capable of altering the host's metabolism sufficiently to induce disease resistance.

SUMMARY

The chemotherapeutic activity of various plant growth-regulators was investigated. 2,4-Dichlorophenoxyacetic acid, α-naphthaleneacetic acid, 2,3,5-triiodobenzoic acid, β-naphthoxyacetic acid, and indole-3-acetic acid reduced the Fusarium wilt disease of tomato.

The chemotherapeutically active plant growth-regulators were relatively poor fungitoxicants *in vitro*.

The chemotherapeutic plant growth-regulators induced changes in the metabolism of the host. Symptomatic of a modified metabolism were a) formative effects b) reduced plant weight and c) a decrease in the reducing sugar content of the host tissues.

The chemotherapeutic activity of the plant growth-regulators was directly related to the interval between inoculation and treatment, the chemotherapeutic activity becoming more pronounced the longer the time that inoculation followed treatment.

The mode of action of the plant growth-regulators as chemotherapeutants is discussed.

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CONTROL OF DISEASE BY HEAT-CURING AND DIPPING GLADIOLUS CORMS. I. WOUND PERIDERM AND THE EXTENSION OF LESIONS [1]

J. G. Bald

One of the usual methods for controlling corm-borne diseases of gladiolus is dipping the corms in fungicidal suspensions or solutions before planting. When applied to gladioli in southern California it has not given consistent results, probably because the final effects of dipping depend on interactions between the host plant, pathogenic fungi, fungicidal materials, and the environment. The immediate object of the work reported in this and 2 following papers was a study of such interactions. The factors considered were: 1) the condition of the corms before treatment, and their natural barriers against invading pathogens; 2) the effect of curing at various temperatures on the condition of the corms; 3) the effects of preconditioning the corms, dipping, and environment on the progress of fungus rots in the corms after planting; 4) the specific effects of fungicides on the pathogens or types of lesion; 5) the effects of treatment on yield. In contrast to ordinary field trials least stress was placed on yield, most on the intermediate stages in the development of lesions and growth of the plant.

The section of the work included here is that related to the effects of heat-curing gladiolus corms. The experiments were designed to discover and test curing methods that encouraged the gladiolus plants' own defenses against invasion by pathogens.

METHODS.—Periderm layers forming at the line of division between parent corms, drying leaf scales, or flower stems and the new corms were examined. Studies were made after harvest on the effects of temperature and, to a slight extent, of humidity on the curing of corms and the development of lesions, by holding healthy and diseased corms in constant temperature cabinets at temperatures between 40°F. and 95°F.

The corms submitted to various treatments were then rated according to the extent and severity of lesions, both those visible on the external surface after the husk was peeled off, and those that became evident internally after a cut was made through the corm. The lesions were mostly of the kinds associated with infection by *Fusarium oxysporum* f. *gladioli* (Massey) Sny. and Hans., and *Botrytis gladiolorum* Timmermans (4). Isolations of these 2 organisms from characteristic lesions confirmed this association.

WOUND PERIDERM AND CUTICLE.—Regions of a newly harvested corm that must be protected by the development of wound periderm layers are the junctions of the sheathing leaves with the corm, the base of the old flower stalk (if a flower has been produced by the parent plant), and the junction between the old and new corms. Of these the last was studied in some detail.

Periderm formation was initiated between the parent corm and the new corm growing above it by secondary thickening of the horizontal walls of parenchymatous cells adjacent to the exhausted tissues of the parent corm. No systematic tests were made to determine whether the material deposited in the walls was lignin or suberin, but Artschwager and Starrett (1) referred to the material in the walls of gladiolus wound periderm as suberin. The vertical walls of the same cells appeared thin and wavy in outline and did not readily accept histological stains. The ease with which the vertical walls were ruptured when the old and new corms were pulled apart suggested that these cells represented a plane of weakness in the tissues as well as the final plane of division between the living storage tissues and the senescent tissues of the old corm. Above this layer of cells within the new corm, a rapid division of cells by horizontal walls initiated the formation of a phellogen layer similar to that forming beneath cut or injured corm surfaces (1). Experimental corms at this stage were compared with corms reported by growers to be ready for cleaning. The experimental and commercial corms had similar cleavage planes and developing phellogen.

Samples of experimental corms were cleaned by the usual growers' method. In most instances, the old corm and roots were readily stripped away from the young corm; the plane of weakness through the layer of cells above the original suberin encouraged a clean break but exposed an area of moist unprotected tissue. If the phellogen had not yet developed very far or was partly torn away with the old corm, living unprotected parenchymatous cells were exposed to the effects of rapid water loss, and to the risk of invasion by more or less pathogenic organisms. The exposed tissue differed little from newly cut or wounded tissue in the body of the corm. Further development of a periderm layer accorded with Artschwager and Starrett's descriptions of wound periderm formation. The effects of temperature and humidity were in the main as described.

At temperatures between 55°F. and 95°F. a normal corm that was not cleaned still formed periderm at the junction with the parent corm. If cleaning was delayed, it often became possible to expose a basal plate fully protected by periderm by stripping away the old corm.

The periderm on a well-cured corm was found to be impermeable to methylene blue in aqueous solution and resistant to invasion by pathogenic organisms (5, 6). The suberin or lignin, deposited in the walls of the more distal periderm cells, was very dense, and fully accounted for this impermeability (Fig. 1, A). Barriers forming below 50°F. before detachment of the parent corm consisted of only a few rows of rectangular cells with thin walls, bounded by several

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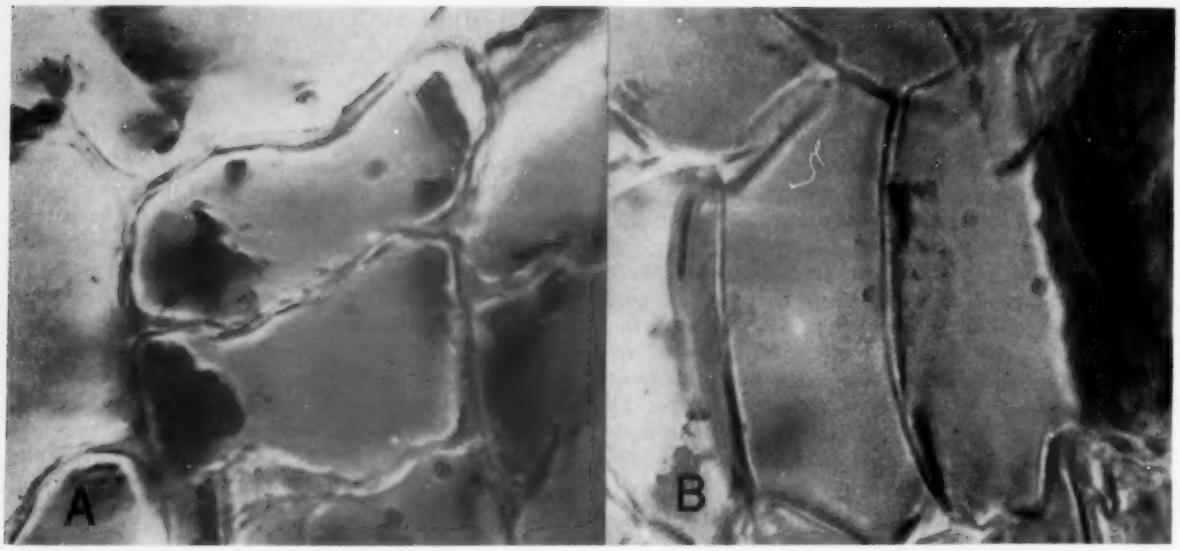

FIG. 1. A) Cells from the distal portion of the basal plate of a gladiolus corm cured at 95°F., showing secondary thickening of the walls; B) Abortive periderm at the base of a corm held at low temperature after harvest. No lignification nor suberization. There is a layer of crushed cells (Right) at the division between old and new corms.

layers of crushed cells (Fig. 1, B). Surfaces on which such abortive periderm had developed remained for some time permeable to methylene blue and liable to penetration by pathogenic organisms.

Periderm formation at the junction of leaves and flower stalks was not studied from its initiation, but cross sections of these regions in properly cured corms readily showed its presence. In normal corms periderm formation occurred beneath the flower stem fairly close to the upper surface of the corm. If organisms had attacked the base of the flower stems, secondary thickening of the cell walls might be incomplete, or periderm might occur some distance below the corm surface, leaving a hollow of greater or less depth at the base of the old flower stem. The depth of this hollow was also affected by the size, shape, and variety of the corm.

Another anatomical effect of proper curing was first noticed in material from a later series of trials.[2] One lot of corms was placed directly in storage at 40°F. several days after they were dug. The cuticle varied in thickness and density. After 2 months an excessive amount of water had been lost from the corms, and some starch had disappeared from the storage cells nearest the epidermis. On corms that were cured at 95°F. for 2 weeks before storage at 40°F., the cuticle was often thicker and invariably more uniform in thickness and texture. Starch filled the parenchymatous cells closer to the surface of the corm than in the noncured series, and was present even in the single-layer epidermis. Such corms retained their condition in storage for long periods of time.

CURING GLADIOLUS CORMS.—In southern California corms are often cured simply by stacking them in open trays in the field; the temperature of curing depends on the season and the current weather. The basic reason for curing, apart from drying the corms after harvest, is to encourage the corms' defenses against invasion by pathogenic organisms, and against undue loss of water during prolonged storage.

So far, the best temperature for artificial curing has been 95°F. (2, 3). Both experimentally and in large-scale commercial trials, corms placed in curing chambers immediately after digging were ready to clean in 6 or 7 days. After cleaning and return to the curing chambers they formed adequate cork barriers in about 4 days and were ready for storage after a few days more, a total curing period of 2 weeks. An additional week at 95°F. or stacking in an open shed might slightly improve the condition of the corms for storage. Through a range of temperatures from 90°F.–55°F. the curing process was adequate but progressively slower. At 65°F. the corms were ready for cleaning in about 3 weeks and required 10 days to 2 weeks longer for periderm formation. At 55°F. the total period was consistently longer than 6 weeks. Below this temperature there was a rapid lengthening of the period and a loss of efficiency in curing. At storage temperature, i.e., 40°F., curing was so inadequate that the corms lost water, became discolored or pigmented, and were exceedingly susceptible to storage rots.

The results from varying the humidity were not so clear-cut. Humidities around 80 per cent saturation, the established lower limit for optimum suberization (1), gave excellent results; but *Penicillium* spp. sometimes developed saprophytically on the basal scars of the corms after cleaning. Whether the *Penicillium* spp. had any ill effects was not determined. In any case, they may be satisfactorily controlled by use of

[2] Material provided by Mr. C. N. Roistacher, formerly of this department.

a mild fungicide, such as Spergon (tetrachloro-parabenzoquinone or Arasan (thiram). At lower humidities there was often too much water loss and shrinkage, but the old corms were more easily detached at cleaning time. ,

Heat-curing Corms and the Extension of Lesions.—The curing process was most easily followed by testing the proportion of the corms from which the parent corms and roots could be broken away. This proportion was low after harvest, before the parent corms were totally exhausted and dry; it rose to a maximum corresponding with the full development of the cleavage planes between old and new corms, and fell again as periderm formation progressed. Thereafter the proportion of corms from which the parent corms could be broken away reached a fairly uniform level characteristic of the sample being tested. Generally, from then on considerable force had to be used in pulling away the dried corms and roots.

The number of corms that broke away cleanly after suberization was well advanced was usually chosen as an estimate of effective curing, because (a) the condition of health or disease by that time was determined, whereas, earlier, organisms might or might not have had time to penetrate past the periderm barrier from the old corm, and (b) subsequent infections were less likely to occur, upsetting the estimates of disease carried in the corms.

A description of 2 representative trials will contain most of the relevant information about the immediate effects of curing on corm lesions. In addition there were 4 other trials primarily designed to study the same phases of the curing process.

A) *Trial 1*—The object of the first trial was to test the effect of curing, begun immediately after digging, on the balance between periderm formation and the development of lesions. Corms cured at a temperature above the optimum for the growth of *F. oxysporum* (95°F.) were compared with corms cured at temperatures favorable for *F. oxysporum* (85°F. and 65°F). The gladiolus variety was Bagdad; humidity was maintained above 80 per cent; and samples consisting of 24 corms were periodically taken for examination from each curing cabinet. Sampling began after 13 days curing, which allowed the completion of periderm formation at 95°F., and very nearly at 85°F. After 13 days at 65°F. corms had not reached the stage at which the parent corms could be broken off cleanly at the basal plates. Sampling continued until the 33rd day, when the corms cured at 65°F. had nearly completed periderm formation.

Samples were divided into (a) corms that broke cleanly at the basal plates (no residue) and (b) those on which remnants of the parent corms remained attached to the basal scars (residue). All were examined for internal and external lesions of Fusarium or Botrytis disease. The majority of corms with clean basal scars were free from obvious lesions, both external and internal. The two curing temperatures comparable on the time scale, 85°F. and 95°F., gave nearly equal

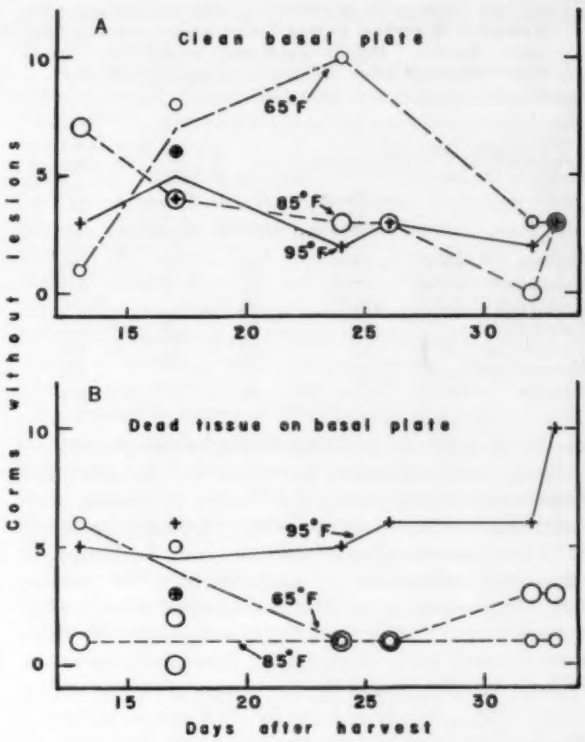

FIG. 2. A) Number of corms per sample of 24 that stripped, leaving a clean basal plate, and had no lesions; B) number of corms that had residual tissue from the parent corm left on the basal plate, but had no lesions.

numbers of corms with clean basal scars and no lesions (Fig. 2, A). Amongst the corms that had residual tissue on the basal scars, those cured at 95°F. had fewer lesions than those cured at 85°F., and there were thus more symptomless corms after curing at 95°F (Fig. 2, B). Curing at 95°F. apparently checked penetration by pathogenic fungi derived from the parent corms.

Results with the series held at 65°F. illustrated the progress of curing as well as the final result. Figure 2A shows the numbers of corms cured at 65°F. that had basal scars without residue and no evident lesions. By the 24th day the numbers had increased to a maximum, representing the full development of cleavage planes between old and new corms. At that stage, a corm already penetrated by Fusarium might have a clean basal scar after the parent corm was broken away, although the tissue was sometimes slightly discolored. Hence, the maximum did not represent the number of corms free from infection through the basal scar. By the 32nd and 33rd days the number of corms without residue or lesions had fallen to the same level as for the high temperature series.

There was also some variation with time in the numbers of corms cured at 65°F. that had residues of tissue from the parent corms, but were free from evident lesions (Fig. 2, B). The numbers began at the higher level of the 95°F. series, but fell to the level of

TABLE 1.—*Classification of gladiolus corms at cleaning, after treatment at various curing temperatures—variety Margaret Beaton. Before treatment, corms were taken from trays left after digging in a commercial field*

Curing Temperature		Percentages			Total Corms Examined
		Basal Scars		Corms	
		No residue	Residue	rotted	
Cabinet	95°F.	14	79	7	543
Cabinet	90°F.	20	76	4	521
Cabinet	85°F.	29	64	7	510
Cabinet	55°F.	50	49	1	331
Greenhouse	70°F.	42	52	6	159
Headhouse	55°F.	42	51	7	176
Outside	50°F.	53	46	1	156

the 85°F. series as periderm formation progressed.

Thus, when allowance was made for the difference in period of curing among the 3 series, the results were consistent. There was a balance between periderm formation and invasion of the new corms by Fusarium, which was influenced by temperature. The balance was weighted in favor of the pathogen when curing temperatures of 85°F. and 65°F. were used. At these temperatures, particularly 85°F., Fusarium grew rapidly both in culture and in the tissues of wound-inoculated corms. In corms cured at 95°F. the balance was weighted in favor of protective periderm formation by the host.

B) *Trial 2*—The second experiment differed from the first in that pathogens had the chance to develop on the corms after they were dug and before heat-curing began. About 3000 Margaret Beaton gladiolus corms were taken from a commercial field. They had been stored in the open in stacked trays for about 3 weeks after they were dug. The weather during that period was intermittently wet, and temperatures were mostly within the range 40°F.–60°F.

The corms were severely infected with Fusarium and Botrytis. Samples of about 500 were kept on trays in cabinets at high humidity, mostly over 80 per cent, and temperatures of 95°F., 90°F., 85°F., and 55°F. Other trays of 150 to 200 corms were kept in the greenhouse (minimum temperature 60°F., mean about 70°F.) in an unheated headhouse (mean temperature around 55°F.), and in the open (mean temperature around 50°F.). After 8 days the corms were cleaned and returned to the cabinets. During cleaning they were divided into 1) those from which the depleted parent corms and roots pulled away completely leaving a clean basal scar (no residue); 2) those on which dead tissue from the parent corms remained on the basal scars (residue); 3) corms that were largely or wholly rotten. Percentage figures for the 3 categories are given in Table 1.

The advantage of curing at lower temperatures suggested by these figures was apparent, not real. As in the previous experiment, many of the corms held at lower temperatures that were easily cleaned had discolored tissue on the exposed basal scar. This indicated that pathogens had already invaded some of the new corms.

A week later corms with and without residue in each curing treatment were rated for internal and external lesions (Tables 2, 3). Again there was a significant association between the presence of residue and penetration of pathogens through the basal plate. Many corms with residual tissue on the basal scar had extensive internal and external lesions. However, this association was confined to the corms cured at constant temperatures, and did not apply to the series cured at the lower fluctuating temperatures. In the latter series, lesions were equally numerous and extensive on corms with and without residual tissue on the basal scars. The main point of contrast between this and the previous experiment was that there were no significant differences among the higher curing temperatures (Table 2). When curing was begun immediately after digging, as in Trial 1, fewer lesions developed at 95°F. than at 85°F. When curing was begun some time after digging, as in Trial 2, the corms meanwhile being subjected to relatively low temperatures, the initial advantage of curing at 95°F. was lost. Other experiments confirmed the advisability of beginning high temperature curing as soon as possible after digging.

Some other results of this trial were: curing at a constant temperature of 55°F. reduced the extension of

TABLE 2.—*Samples of Margaret Beaton gladiolus corms (see Table 1) rated for presence of lesions. Square roots of original ratings used for analysis. Below are means of 3 replicate square root values.*

Mean curing temperature in degrees F.	Curing Temperature							
	Constant temperature				Fluctuating temperature			
	Basal scars with:				Basal scars with:			
	No residue		Residue		No residue		Residue	
	Lesions		Lesions		Lesions		Lesions	
	internal	external	internal	external	internal	external	internal	external
95	0.47	1.37	3.17	2.97				
90	0.73	1.33	2.83	2.40				
85	0.57	0.47	2.93	3.07				
70					1.60	2.60	1.60	2.53
55	0.57	3.00	3.67	3.67	2.60	3.00	3.37	3.60
50					2.97	3.43	4.03	3.67

Least significant difference between means of total lesion ratings, internal plus external, for 3 replicates = 2.96.
Least significant difference internal versus external lesion, means of 3 replicates = 0.95.

TABLE 3.—*Analysis of variance; square roots of original ratings; Means of 3 replicates are in Table 2*

	d.f.	S.S.	v.	
Total between corms	41			
Curing treatments	6	35.0967	5.8495	S
Basal scars residue	1	38.2247	38.2247	S
C.t. × B.S.	6	15.0605	2.5101	S
Error	28	21.5381	0.7692	
Total for internal and external lesions on same corms	42			
Internal v. External lesions	1	3.9762	3.9762	S
C.t. × I. v. E.	6	4.0490	0.6748	N.S.
B.s. × I. v. E.	1	2.7681	2.7681	S
C.t. × B.s. × I. v. E.	6	3.8833	0.6472	N.S.
Error	28	9.0034	0.3216	

internal but not of external lesions on corms without residue, whereas the higher constant temperatures reduced both. The treatment that induced the most rapid expansion of lesions was exposure to outdoor winter conditions somewhat similar to those in which the corms had been placed after digging (mean temperature about 50°F.; Table 2).

Secondary Results of Heat Curing.—Some secondary physiological effects of heat-curing gladiolus corms were observed during these trials. The condition of corms grown and dug in cool weather when they had no true dormancy was obviously affected. At 85°F. the immediate growth of root initials and buds was encouraged. On the other hand, premature sprouting of roots on the corms and resultant loss in quality of crops grown from them, were sometimes prevented by the 95°F. curing treatment (3). In 1 unpublished experiment with cormels, a parallel effect, retardation of germinability, was obtained by heat-curing at 95°F. and by soaking in a solution of indol-3-acetic acid. Although in that instance, reactions to the 2 treatments were superficially similar, it did not follow that the action of heat on the cormels was the same as that of the hormone treatment.

In another experiment a sample of dormant corms was first heat-cured for 4 weeks and then placed at 40°F. for 6 weeks. This sample sprouted more quickly than checks held at room temperature, and much more quickly than corms heat-cured and then held at room temperature. It seems likely from these and other results that heat curing of gladiolus corms might be adapted to a program for controlling their physiological condition, in addition to the control of disease.

DISCUSSION.—In the period since the experiments mentioned above were completed, heat curing of gladiolus corms has been repeatedly tested on a large scale by gladiolus growers in southern California. Conditions in the commercial curing chambers have often varied widely from the ideal of 95°F. and 80 per cent humidity, but the farmers who have heat-cured gladiolus corms have claimed in most instances a great improvement in condition and a striking reduction in storage rots and the carryover of disease. There have been sufficient exceptions to this rule to indicate the need for further work, particularly on the physiology of heat curing.

The following is a program for large scale curing worked out in cooperation with a grower in San Diego County. The corms were dug by machine, loaded into trays, carted to the curing chamber, and stacked inside. Temperatures of about 95°F. were maintained and the air was circulated by fans. Sufficient fresh air was drawn in through a vent to prevent condensation on the corms, but humidity around the corms themselves was over 80 per cent. After a week, the corms were cleaned and graded by machines that had previously fallen into disuse because they caused a degree of bruising that tended to increase the development of rots in storage. The corms were then dusted with Arasan or Spergon. The curing and dusting neutralized any bruising from machine operations and protected the basal scars from infection by Fusariums and other pathogens (4). The corms were returned to the curing chamber and temperatures were maintained at 95°F. for 5–8 days. At this stage they were ready for storage, but generally they were left at air temperature until there were signs of breaking dormancy; then they were placed in the cold room at 40°F.

SUMMARY

The corms' natural barriers against infection by pathogens, periderm and cuticle were studied after treatment of the corms at different curing temperatures. The best treatment tested for developing periderm and cuticle resistant to infection was curing corms immediately after digging at a temperature of 95°F. This temperature also checked the advance of pathogenic fungi into the tissues.

Delay in heat curing until corms were in condition for stripping off the old corms and roots dissipated the initial advantage of curing at 95°F., probably by allowing disease organisms to penetrate the tissues of the new corms before periderm was formed.

A program for large-scale use of heat curing was worked out in cooperation with commercial gladiolus growers.

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CONTROL OF DISEASE BY HEAT-CURING AND DIPPING GLADIOLUS CORMS. II. INCIDENCE OF LESIONS [1]

J. G. Bald

A necessary preliminary to the study of dipping gladiolus corms in fungicidal materials was the identification of the causal organisms in at least some of the lesions on the corms. This was undertaken by field and laboratory experiments and observations, to distinguish the various effects of dipping on the development of different corm-borne diseases (2). The results of the work on lesions are included in the following paper.

FUSARIUM YELLOWS AND BASAL ROT.—In any large collection of gladiolus varieties infected with Fusarium diseases it has not been found possible to maintain on a symptomological basis the division between Fusarium basal rot and Fusarium yellows (7). On different varieties a gradation was found between the 2 symptom types (Fig. 1, A-D). There is no doubt that strain differences exist among these Fusaria (5), but there is little doubt that there are varietal differences in reaction of gladioli to similar strains. The problem of variation and specificity can be solved only by large-scale cross inoculation studies with representative gladiolus varieties and representative isolates of Fusarium.

Fusarium isolates were obtained from gladiolus varieties with typical basal rot symptoms and from other varieties with yellows and intermediate symptoms (Fig. 1, A-D). In culture all Fusaria obtained consistently and predominantly from diseased corm tissues fell within 1 range of morphological types. Typical single spore cultures from basal rot, yellows, and intermediate infections were submitted to Dr. W. C. Snyder for identification. He placed all the cultures in the species *Fusarium oxysporum* Schl. As these Fusaria were obtained from active lesions, and some similar isolates were shown by inoculation tests to be pathogenic on gladiolus corms, the strains causing basal rot, yellows, and intermediate symptoms on gladioli in southern California have been provisionally grouped under the name *F. oxysporum* f. *gladioli* (Massey) Sny. and Hans.

During this work many hand sections were taken from known or suspected corm lesions of *F. oxysporum* f. *gladioli* in various stages of development. Stab inoculations of cormels provided material for some of the more active lesions. Advancing mycelium was mainly intercellular in the storage tissues of both corms and cormels. Intracellular mycelium was often present some distance behind the advancing margins of lesions. Generally it consisted of occasional hyphae passing around and between still intact starch grains within parenchymatous cells; more rarely the intracellular hyphae were numerous and ran in all directions. As the lesions aged, much of the intercellular mycelium disappeared, leaving ragged spaces in the cell walls where it had been. In lesions that were no longer active it was difficult to find mycelium, although colonies of bacteria were occasionally present. Penicillia often occurred externally on the dead tissues. Under some conditions single-celled Fusarium chlamydospores were found, particularly in the disintegrated tissue of hollowed out areas.

These histological details have been useful in diagnosing Fusarium rots. Intercellular advance of Fusarium mycelium is constantly associated with basal rot symptoms, and is not so evident when symptoms above ground are of the "yellows" type.

As might be expected, the greatest development of Fusarium symptoms in gladioli grown along the coastal belt of southern California occurs during summer. However, corm lesions from which *F. oxysporum* can be isolated are found during winter, and during all seasons Fusarium may be carried on and in corms without causing obvious symptoms. Isolations from apparently healthy tissue above the basal scar of corms from storage, and from barely discolored tissue at different sites in almost unblemished corms, have given cultures of *Fusarium*. Isolations from the dried husks of corms without serious blemishes on the surface have also yielded *Fusarium*.

In 1 experiment involving 1152 Picardy corms, each corm was dehusked, sterilized, and indexed by plating tissue on potato-dextrose agar. Later 850 of the corms free from all visible lesions and failing to yield a pathogen in culture, were planted in sterilized soil.

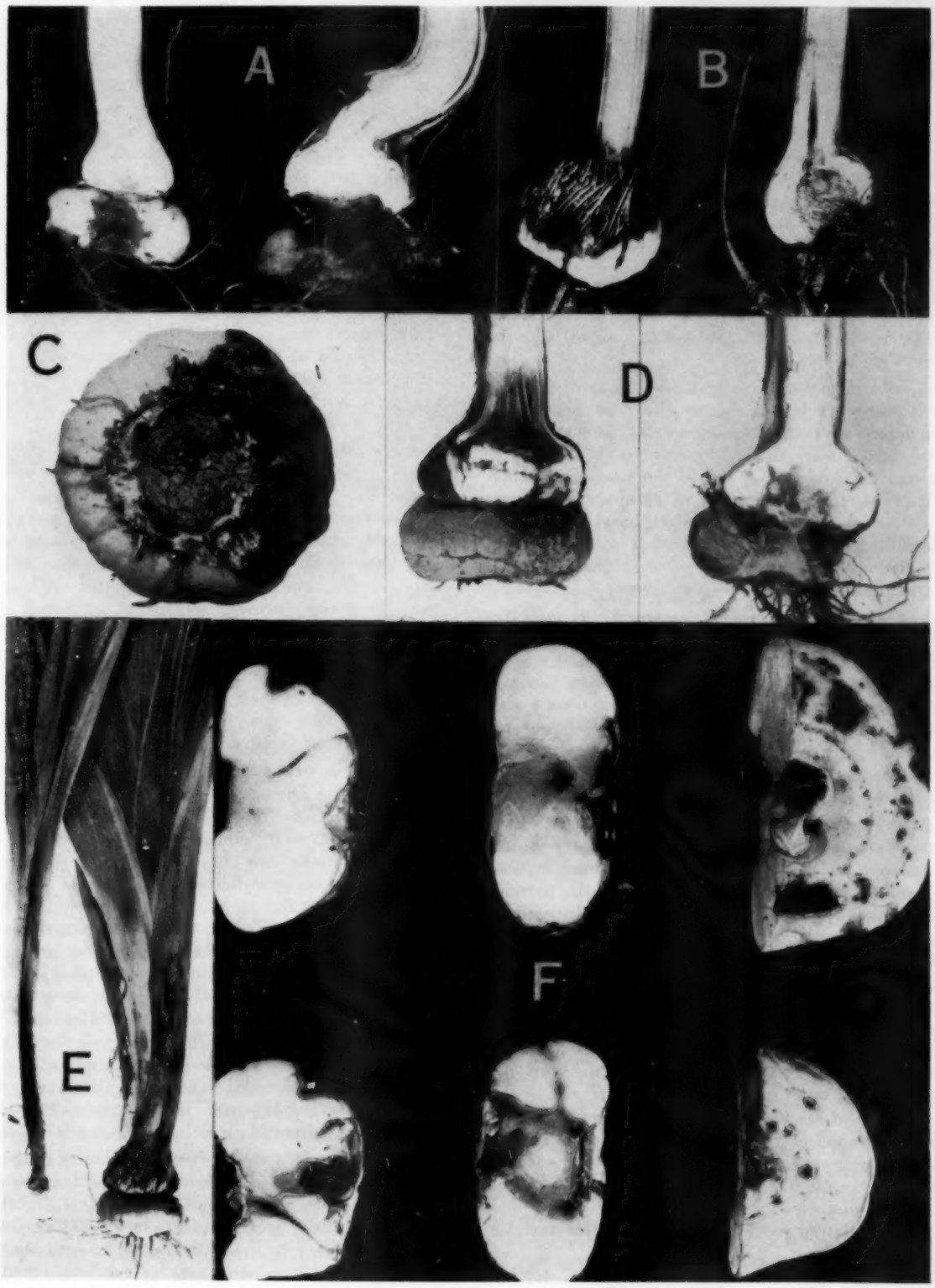
[1] Accepted for publication December 8, 1952.

Thanks are due to Mrs. Lily H. Davis for making numerous isolations and cultures of the organisms mentioned in this paper.

FIG. 1. A-D, Fusarium disease of gladiolus. Plants of 3 varieties growing in the same field; A) Sections through base of 2 Picardy gladiolus plants with symptoms of basal rot. Parent corms rotting. On right, rot involves new corm, but there is no vascular invasion; B) Variety Corona, infected by Fusarium. Symptoms intermediate between basal rot and the vascular invasion characterizing Fusarium yellows; C) Variety Picardy, basal rot on underside of corm; D) Variety Snow Princess, symptoms of Fusarium yellows. Left, external symptoms, right, vascular invasion from old to new corm without immediate rotting of parenchymatous tissues; E) Symptoms of *Sclerotinia gladioli* on 2 adjacent gladiolus plants from the field. Left, infection of long standing, right, infection recent; F) Lesions of *Botrytis gladiolorum* on two gladiolus varieties. On Lady Jane (above) external lesions (right) are large and superficial, penetration from base of flower stalk (center) is checked by periderm, and penetration along vascular bundles (left) is rare. On Helen Mayne (below) external lesions (right) are small, penetration along vascular bundles from flower-stem bases, leaf bases, and buds (center) may rapidly involve the vascular areas around the core, or (left) pass through the corm from above to the basal plate.

Three per cent had foliage symptoms of Fusarium basal rot and other plants had Fusarium lesions on the corms.

These and similar results explain a common experience when gladiolus corms are moved from a cool to a warm environment, e.g., from the Pacific North-



west into southern California. The corms may have little evidence of Fusarium infection when planted, yet the resulting crop grown during summer in clean soil may be badly diseased. Similarly, gladioli grown locally during the cooler seasons and used for summer planting may become far more seriously diseased than the condition of the parent corms would indicate. Apparently Fusarium may be carried through 1 or more successive plantings without causing a serious disease. When the plants are subsequently grown in an environment favorable for *F. oxysporum* full development of symptoms becomes possible.

F. oxysporum was in one instance also carried through a winter planting of gladioli in the desert. Relatively cool daytime temperatures coincided with the growth of the daughter planting during the following winter, and a severe but atypical disease developed. The root systems were attacked, superficial lesions developed on the base of the corms, and the plants were badly stunted.

By experiments of various kinds it was also established that when lesions due to *Fusarium* were found on a parent gladiolus corm at digging, internal or external lesions were likely to occur on the new corm that had developed from it. In some instances when corms were cleaned, remnants of tissue from the parent corms remained attached to the basal scars. The probability was high that *Fusarium* or some other pathogenic organism had penetrated through such basal scars into the new corms (1).

The accumulated evidence summarized here indicates that lesions on the corms may be a positive indication of Fusarium, but that considerable Fusarium inoculum may be present on corms relatively free from lesions. In the control of Fusarium, fungicidal treatments may be advisable on corms apparently free from serious infection, and on corms planted where Fusarium is not generally a serious disease or during periods unfavorable for the full manifestation of symptoms.

LESIONS DUE TO BOTRYTIS GLADIOLORUM TIMMERMANS.—Isolations from Botrytis corm lesions were not so numerous as the isolations from Fusarium lesions. They served mainly as a check on diagnosis, particularly of the less obvious or atypical lesions which might be concerned in the carryover of Botrytis infection. A study was made of the disposition of Botrytis lesions on an aggregate of several hundred gladiolus corms of several varieties harvested during winter. The most frequent points of origin of lesions on the larger corms appeared to be the leaf scars and the bases of flower stalks (Fig. 1, F). On many field-grown plants the progress of infection into the corms was traced by the discoloration of the tissues from the cut ends of flower stalks downwards, or by vertical discolored streaks through the encircling leaf bases. The presence of Botrytis in the discolored foliage tissues and in lesions or slightly discolored regions inside the corms was checked by plating pieces of tissue on potato-dextrose agar.

Microscopic examination was used as a further check. In both foliage and corm tissues Botrytis mycelium was often subcuticular (Fig. 2,A), and in superficial lesions might not penetrate noticeably between the living cells. Where discoloration followed the vascular bundles, long stout hyphae were found running parallel with the bundles along the walls of parenchymatous cells rather than internally along the vessels. As breakdown of these tissues occurred, Botrytis mycelium ramified and penetrated the vessels. Botrytis often grew superficially between enfolding leaf bases, particularly under wet conditions. This sort of mycelial growth probably accounted for numerous infections in vegetative buds of corms held during winter on piled-up trays in the field. Lack of dormancy under cool conditions allowed some development of vegetative buds on the corms immediately after digging. The incidence of bud lesions suggested infection from the enfolding leaf scales, either by penetration into the meristematic tissues, or at the junction between meristematic tissues and the storage tissues of the corm. Having penetrated, the mycelium frequently progressed along the vascular bundles leading inwards from the buds (Fig. 1,F).

Considerable varietal differences were found in the reaction of corms to invasion by Botrytis. On the corms of some, e.g., Lady Jane (Fig. 1,F, above), large dark brown surface blemishes occurred and there was relatively little penetration of the tissues. Mycelium was mainly subcuticular, several layers of cells were killed by toxic action, and internally a periderm layer was formed. Vascular invasion was often restricted. In other varieties, e.g., Helen Mayne (Fig. 1,F, below), vascular bundles and parenchymatous tissues were readily invaded, and the core often rotted out. Water-soaking of tissues at the margin was a regular feature of Botrytis lesions expanding through storage tissues in this and simliar varieties.

OTHER TYPES OF LESIONS.—Of the corm-borne diseases common in other areas, lesions of hard rot (Septoria) were not found in southern California, and those of bacterial scab were quite common but occurred in serious proportions only in certain restricted areas. They were not of importance in the present studies. Lesions of dry rot due to *Sclerotinia gladioli* Drayton were more frequently found. Dry rot was serious in isolated areas of soil during the cooler months of spring and early summer, and isolated infected plants could be found at almost any time of the year in many gladiolus stocks. The generally unsuitable environment of southern California seems to have checked the large-scale development of this disease, although it is potentially serious in soils where gladioli have been grown for a number of years. In some instances it has eliminated the culture of gladioli from individual fields.

Dry rot lesions in stored corms were similar to those described by Moore (6). They had some features in common with scab or Botrytis lesions (Fig. 1,E, 2,B). On the leaf bases of growing plants they could often be distinguished from both by their dark brown, almost black color when they were actively expanding under

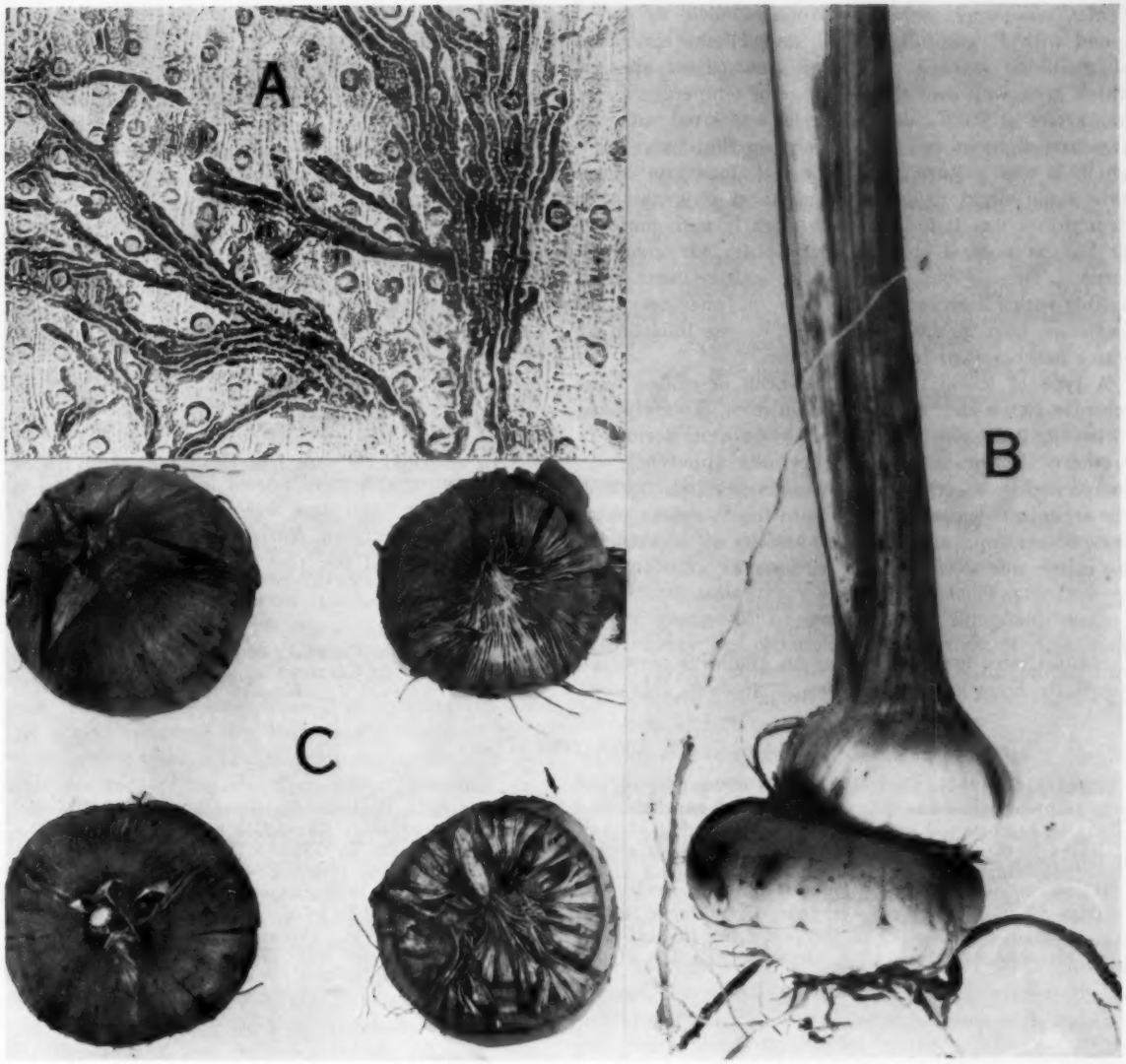


FIG. 2, A) Subcuticular mycelium of *Botrytis gladiolorum* in leaf epidermis of a gladiolus plant; from the edge of a large advancing lesion; B) lesions of *Sclerotinia gladioli* on base of plant after removal of outer shredded leaves and corm husk; C) right, shredding of corm husks due to Rhizoctonia *solani*, left, normal corms.

humid conditions. Subsequent development of sclerotia often put the diagnosis beyond question. The microscopic appearance of lesions on the leaf bases was somewhat like that of Botrytis, but the hyphae were more variable in size.

Another pathogen regularly found on some stocks of gladioli was *Rhizoctonia solani*. In mature corms Rhizoctonia caused a shredding of the corm husks (Fig. 2,C) and of the outer basal sheathing leaves, without causing much damage to the growth of the plant. On stored corms it was often difficult to find evidence of Rhizoctonia hyphae until the tissues from the less seriously shredded husks were cleared and examined under the microscope. Hyphae similar to runner hyphae were then found inside the dead tissues. They gave off typical branches, constricted at the point of junction and septate near the constrictions. If affected corms were placed in a moist chamber, Rhizoctonia runner hyphae often appeared on the corm surfaces. Rhizoctonia was readily obtained in culture from surface-sterilized pieces of affected husks.

The shredding of the corm husks due to this fungus was part of the combination of lesions on stocks of corms used in several of the field trials, but there was little evidence of it on the corms used for greenhouse bench trials.

Microscopic examination also revealed Rhizoctonia mycelium on cormels from infested corms. *R. solani* was found causing a serious neck rot of small gladiolus plants growing from cormels (4).

From many minor lesions and blemishes on corms, as well as from many Botrytis lesions, several types of

Penicillium were isolated. None seemed to correspond with *P. gladioli* McCull. and Thom., the cause of gladiolus storage rot. One *Penicillium* species, which grew well over a wide range of temperature and was active at 95°F. was definitely associated with the presence of blemishes and lesions on the corms from which it was cultured. Stab inoculations into corms gave some slight evidence of pathogenicity. Another *Penicillium* was found that was slightly pathogenic at 40°F., the normal storage temperature for gladiolus corms. These *Penicillium* spp. in culture were compatible with *Fusarium* and *Botrytis*, and they may have had some part in the extension of lesions initiated by active pathogens or by the root mite.

A type of lesion that has not been described until recently (3) is that due to the root mite, *Rhizoglyphus rhizophagus* Bks., which appears to be quite serious in southern California. Mite injury was apparently accompanied by a certain amount of injury from secondary organisms such as *Fusarium* of the *F. roseum* type, *Penicillium* spp., and bacteria, and by an increase in the injury due to established pathogens.

SUMMARY

Studies were made of lesions on gladiolus corms infested by Fusarium *oxysporum*, f. *gladioli* (Massey) Sny. & Hans., *Botrytis gladiolorum* Timmermans, and other organisms.

The advance of Fusarium through storage tissues was mainly intercellular. The absence of visible lesions on the corms did not connote absence of infestation by Fusarium. Inoculum was often carried on or in the dried husks.

The climatic and seasonal incidence of Fusarium lesions on the corms did not always correspond with the degree of corm infestation, as revealed by growing a stock of corms under conditions favoring Fusarium disease.

Botrytis corm lesions appeared often to be initiated at buds and leaf scars. In some varieties Botrytis lesions were relatively superficial, in others hyphae advanced more readily along vessels and through storage parenchyma.

The nature and incidence of lesions associated with the following organisms were also discussed: *Sclerotinia gladioli* Drayton, *Rhizoctonia solani* Kühn, Penicillium spp., and the root mite, *Rhizoglyphus rhizophagus* Bks.

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CONTROL OF DISEASE BY HEAT-CURING AND DIPPING GLADIOLUS CORMS. III. DIPPING TRIALS [1]

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Two previous papers (1, 2) described the ripening of gladiolus corms and wound periderm formation, the effects of heat-curing on the balance between the corms' natural defenses and establishment of pathogenic fungi in the tissues, and the nature and incidence of corm lesions. The present paper deals with the use of fungicidal dips, and interactions between (a) their efficiency in controlling various disease symptoms, and (b) the factors introduced by pretreatment of the corms and subsequent conditions of growth. The study of interactions was made to gain information on the reasons for variable results obtained by the use of fungicidal dips, and also on methods of combatting this variability.

The experiments in this section of the investigation included 7 greenhouse bench experiments and 5 replicated field trials, in addition to several growers' trials. In design the bench experiments were similar to the replicated field trials. Although they involved far fewer plants, they gave significant results because the plants were grown in a uniform sterilized soil mix under less variable conditions than exist in the field. The general plan of the work was determined by its exploratory nature. The major experiments and field trials formed an interlocking series in which each phase of one was generally repeated at least once in others, but no 2 trials or experiments were designed to study precisely the same combination of factors.

The gladiolus corms used were in part from stocks grown on the University of California, Los Angeles campus, in part from commercial stocks infected largely with *Fusarium oxysporum* f. *gladioli* (Massey) Sny. and Hans. and *Botrytis gladiolorum* Timmermans. The 2 varieties most used in the greenhouse trials were Margaret Beaton and Picardy; various other varieties were included in the field trials.

The fungicidal materials in the dips were chosen for the contrast in their properties. Those most used were Lysol, ferbam (Fermate), and a mixture of the two. An early greenhouse experiment and 2 field trials indicated that Lysol plus ferbam was moderately effective as a dip against a combination of diseases including those caused by *Fusarium* and *Botrytis*. Under some conditions it was comparable in efficiency with New Improved Ceresan. Two greenhouse bench experiments gave a comparison among 6 carbamates, by themselves and in combination with Lysol. Ferbam yielded more consistent results than the other carbamates and was chosen for use in the most comprehensive experiment, the only one of the greenhouse bench experiments that will be described in detail.

METHODS.--The corms in these trials were from the lot of Margaret Beaton used in the curing trial No. 2 described in a previous paper (1). Samples of corms were taken within 4 curing treatments, 95°, 90°, 85°, and 55°F., from the groups that after cleaning had residual tissue from the parent corms on the basal scars (1, Table 1). There were 4 treatments in the trial: dipped in water (checks); dipped in Lysol; dipped in ferbam; dipped in Lysol plus ferbam. The dipping period was 30 minutes. Ferbam was used at a concentration equivalent to 2 lbs., and Lysol at 2 qt. per 100 gal. of water.

Sixteen corms given the same treatment were planted in each of 64 flats. Half of the flats, including all treatments, were held in the greenhouse, half in cabinets without light at 55°F. The latter were allowed to remain in the cabinets until the more advanced plants, completely etiolated from growth in the dark, were 12–15 in. tall. They were then placed alongside the other group in the greenhouse. The final range of treatments was 4 curing temperatures, 4 dips within each curing temperature, and 2 sprouting temperatures. This made a total of 32 treatments, each represented by 2 flats of 16 corms.

One flat of each pair was sampled twice during the vegetative preflowering stage of growth for a study of the effects of treatments on the condition of the growing plants. The other flats were maintained until growth was complete and the corms were harvested and weighed. The first sampling, of 4 plants per treatment, was made after the etiolated cabinet-sprouted plants had become normal in appearance and growth, the second, of 12 plants, 6 weeks later. The data recorded separately for each plant sampled were: (a) shoot normal, rated 0, shoot spindly or with lesions at the base, rated 0.5, shoot rotted away or failed to emerge, rated 1; (b) a similar classification and rating of the condition of the roots; (c) rot extending through part of the corm, or internal necrosis mainly centered on the vascular bundles around the core—presence rated 1, absence 0; (d) vascular invasion from the basal scar of the type almost invariably associated with *Fusamium*, presence rated 1, absence 0; (e) lesions on the surface of the corm of types generally associated with *Botrytis*, *Fusarium*, *Sclerotinia*, or bacterial scab (the last 2 were minor pathogens), presence rated 1, absence 0; (f) lesions originating at the base of the flower stem primarily due to Botrytis, presence rated 1, absence 0; (g) lesions on the buds or originating in the angles between buds and corm surfaces, presence rated 1, absence 0.

These last 2 were due to field infection with *Botrytis* or to spore-borne infection when trays of corms were stacked in the field after harvest. Because of a similar reaction to treatments, (f) and (g) were bulked. Symptoms types (a) to (e) were largely due

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TABLE 1.—*Dipping trial: tabulation of disease ratings and yields to demonstrate the main effects of treatments, and also interactions between curing temperatures, fungicidal dips, and conditions of sprouting*

Column number		1		2		3		4		5		6		7	
						Ratings, corm lesions:									
Row No.	Treatments	Ratings shoots		Ratings roots		Rots and internal necroses		Vascular invasion from basal scar		Surface lesions		Lesions at buds and bases of old flower stalks		Yield oz.	
		Cab.	Gr.	Cab.	Gr.	Cab.	Gr.	Cab.	Gr.	Cab.	Gr.	Cab.	Gr.	Cab.	Gr.
	No lysol	34	46	46	43	14	34	12	11	12	13	80	89	18	42
1.	Lysol	39	31	57	31	20	20	11	10	14	14	70	74	38	38
	Significant differences[a]	– – *		– * –		– – *		– – –		– – –		– – *		* – –	
	No ferbam	41	38	61	38	16	28	13	15	12	15	80	86	23	33
2.	Ferbam	32	39	42	36	18	26	10	6	14	12	70	77	33	47
	Significant differences[a]	– – –		* * –		– – –		– – *		– – –		– – –		* – *	
	Curing temp.: 55°F.	18	21	23	24	13	22	7	3	8	6	37	27	17	19
3.	85	18	25	27	19	9	15	5	7	4	4	41	40	9	23
	90	19	18	26	21	5	13	3	6	9	10	38	44	12	21
	95	18	13	27	10	7	4	8	5	5	7	34	52	18	17
	Significant differences[a]	– – *		– – *		– – *		– – –		– – –		– – *		* * –	
		No. L.	L.	No. L.	L.	No. L.	L.	No. L.	L.	No. L.	L.	No. L.	L.	No. L.	L.
	Curing temp.: 55°F.	24	15	28	19	19	16	5	5	4	10	36	28	12	24
4.	85	22	21	20	26	16	8	7	5	4	4	41	40	20	12
	90	16	21	21	26	8	10	5	4	10	9	42	40	17	16
	95	18	13	20	17	5	6	6	7	7	5	50	36	11	24
	Significant differences[a]	– * –		– * –		* * *		– – –		– – –		* – *		* * *	
		No. F.	F.	No. F.	F.	No. F.	F.	No. F.	F.	No. F.	F.	No. F.	F.	No. F.	F.
	Curing temp.: 55°F.	20	19	27	20	17	18	9	1	6	8	34	30	12	24
5.	85	22	21	26	20	11	13	5	7	2	6	44	37	13	19
	90	21	16	27	20	10	8	6	3	12	7	47	35	15	18
	95	16	15	19	18	6	5	8	5	7	5	41	45	16	19
	Significant differences[a]	– – –		– – –		* – *		– * –		– – –		* – *		– – –	

[a] Significant or nonsignificant differences, represented by an asterisk or minus sign, refer to the values vertically above the sign. The asterisk or minus sign in the centre of each group of 3 refers to the interaction between factors represented in the cell of the table in which it occurs. Cab. refers to sprouting in cabinets at 55°F., Gr. in the greenhouse at about 70°F., L in row 4 indicates Lysol, F in row 5 indicates ferbam.

to *Fusarium*, although other organisms were probably involved, particularly in (e). The root symptoms (b) were in part associated with early sprouting and injury during handling and storage. The early sprouting was a result of the corms being dug in cold weather and having no true dormancy.

The arrangement of the dipping treatments allowed separate estimates of the effects produced by Lysol and ferbam and the interaction between the two. The rating method was as simple as possible to avoid artifacts and bias in comparisons between different types of symptoms. Ratings for shoots and roots were made only at the second sampling. Comparison of rating values for corm lesions at the first and second sampling indicated similar trends in the data, but the retardation by Lysol of symptom development was more evident at the first rating. Other experiments also suggested that some effects of Lysol were transitory. As no more information was obtainable by keeping separate the values for corm lesions at the 2 harvests, they were bulked.

Detailed analyses similar to those described in a previous paper (1) were made on the ratings for various types of symptoms and on the yields from the duplicate flats. First-order and second-order interactions were examined, as well as the main effects of dipping treatments, curing temperatures, and sprouting temperatures. A probability of .05 was accepted as the level of significance. Results are in Table 1.

Dips containing Lysol.—During the period of vegetative growth there were similar effects of Lysol on disease ratings for shoots, roots, and rots and internal necroses of the corms (Table 1, row 1, columns 1, 2, 3). All 3 groups of symptoms were associated in some degree with Fusarium infection. The effect of Lysol on corm rots and internal necroses seemed transitory, as it was not finally reflected in yield (see below). Lysol also affected the lesions developing from buds and the bases of old flower stalks, which had been initiated by Botrytis (row 1, column 6). Lysol treatment caused a reduction in disease ratings for plants sprouted under warmer conditions in the greenhouse,

but no reduction, sometimes the reverse, for plants sprouted under cooler conditions in the cabinets.

Lysol had no effect on the most characteristic symptom of Fusarium disease, necrosis of the vascular bundles leading up from the basal scars of the corms (column 4).

On the yield of corms (row 1, column 7) treatment with dips containing Lysol produced no significant effect for greenhouse-sprouted plants, and an increase for cabinet-sprouted plants. The effects of sprouting in greenhouse or cabinet were the opposite of what might have been expected from the interim disease ratings. Lysol caused only a temporary delay in the progress of diseases on greenhouse-sprouted plants. In the series sprouted in the cabinets at cooler temperatures some of the pathogens must have had their effect on yield mostly during the later stages of growth. During that period injury must have been less severe on plants treated with Lysol.

In searching for a possible association between the effects of Lysol on pre-flowering disease ratings and its effects on yield, the inconsistencies introduced by conditions of sprouting were first eliminated. Rating values for greenhouse- and cabinet-sprouted corms were bulked. Comparisons of ratings and yield were made between values in the 2-way tables for Lysol versus no Lysol at the 4 curing temperatures (Table 1, row 4). The sums of ratings for roots and shoots, and for lesions developing from the bases of old flower stalks and buds, obtained by adding values in row 4, columns 1, 2, and 6, were inversely correlated with the corresponding figures for yield ($r = 0.907$, odds for significance greater than 99 to 1). Taken singly, the ratings in any of the columns 1, 3, or 6 were not significantly correlated with final yields, and addition to these rating totals of ratings in columns 3, 4, or 5 diminished or eliminated the significance of the correlation. The effect of Lysol on yield therefore seemed to be associated mainly with its effects on the condition of shoots and roots, and on extension of lesions from the buds and the bases of old flower stalks.

Dips containing ferbam.—A regrouping of treatment totals was made into pairs with and without ferbam (Table 1, row 2). The action of ferbam was more straightforward than that of Lysol, although again there were indications that the final influence of the 2 sprouting temperatures on the effects of dipping were not yet evident in the interim disease ratings. Dips containing ferbam reduced significantly the spindliness and rotting of the roots on cabinet-sprouted plants (row 2, column 2), but not on greenhouse-sprouted plants. It did not affect significantly the condition of the shoots, as Lysol did. It reduced vascular penetration of *Fusarium* from the basal scar, mainly in greenhouse-sprouted material (row 2, column 4), which Lysol did not. Consistent with these effects, ferbam improved the final yield of plants from both cabinet and greenhouse-sprouted corms (column 7). Also consistent was an association between the combined ratings for roots and for vascular penetration (obtained from row 5, by adding columns 2 and 4), and the yield (column 7). The correlation coefficient was 0.883 for the 8 pairs of values. The increase in yield of corms from the use of dips containing ferbam was therefore mainly associated with their aggregate effects on the condition of the roots of the plants and their capacity to reduce invasion of *Fusarium* from the basal scars of the corms.

Interaction between Lysol and ferbam.—A study of the interaction between the effects of Lysol and ferbam showed that it was negligible. Lysol and ferbam had different effects on the various phases of corm-borne infection, and they did not noticeably interfere with each other's action. There was slight evidence of interaction in another pair of experiments involving 2 gladiolus varieties, Lysol, and 5 carbamates including ferbam. The interaction appeared to be temporary in its effects on the development of lesions. The available evidence from greenhouse and field trials indicates that the effects of Lysol and ferbam in combination are largely additive. Lysol plus ferbam should, therefore, be a more effective fungicide than either lysol or ferbam alone.

Curing temperatures.—The temperatures at which the parent corms were cured influenced both pre-flowering disease ratings and the yields of plants left to mature. This is revealed in some of the dissected data. Ratings in Table 1, row 3, columns 1, 2, 3, and 4 were added to show the combined effects of curing temperatures and conditions of sprouting on lesions induced wholly or in part by *Fusarium*. Among these 4 groups of lesion ratings were minor variations in reaction to sprouting temperatures, e.g., root ratings for cabinet-sprouted corms tended to be higher than for greenhouse-sprouted corms, whereas ratings for rots and internal necroses were higher on the greenhouse-sprouted corms. On the whole, however, a curing temperature of 95°F. and subsequent sprouting at warm temperatures in the greenhouse reduced expansion of these lesions. The same conditions increased expansion of lesions initiated by *Botrytis* at the base of old flower stalks and buds (column 6). These opposite effects apparently balanced each other, as there was no significant gain or loss in yield from curing at 95°F. and sprouting in the greenhouse (column 7). On the other hand, curing at 85°F., a favorable temperature for growth of *F. oxysporum* f. *gladioli* (Massey) Sny. and Hans., and sprouting in cabinets at 55°F. significantly reduced the final yield (row 3, column 7).

The combined influence of dipping and curing temperatures on disease ratings and yield (row 4) has already been illustrated. Dipping with Lysol plus ferbam gave the greatest increase of yield on plants from corms cured at 55° and 95°F. (row 4, column 7). It must be remembered in comparing the effects of these 2 curing temperatures on this stock of gladioli, that some initial advantage of curing at 95°F. was lost by leaving the corms in the field about 3 weeks after digging.

Secondary organisms in corm lesions.—Lesions originating from Botrytis infections at the base of old flower stalks and at buds (Table 1, row 3, column 6) before the plants flowered were most extensive on corms submitted to curing at 95°F. and to the warm sprouting and growing conditions in the greenhouse (mean above 70°F.). *Botrytis* is a low temperature organism which does not normally grow through corm tissue at temperatures much above 50°F. (4). It ceases growth on any medium at about 75°F. Yet these lesions expanded under conditions unfavorable for *Botrytis* and favoring a high temperature organism. Secondary invaders of the Botrytis lesions probably caused their expansion at high temperatures.

In addition to *Botrytis* and sometimes *Fusarium*, isolations from the inner edges of such lesions gave mainly *Penicillium* spp. and bacteria (2). The bacterial colonies were often similar in appearance to *Bacterium marginatum*. Some organisms of these types grow readily at temperatures between 70°F. and 95°F.

Treatment with dips containing Lysol reduced the extension of lesions originating at the base of flower stems and buds (row 1, column 6); and ferbam failed to do so (row 2, column 6). Of the 2 materials, Lysol probably killed or retarded the secondary invaders of the Botrytis lesions that were capable of extending those lesions under high temperature conditions.

On the other hand, Lysol appeared to encourage the extension of certain corm lesions originating from infection with *Fusarium*. Occasionally this resulted in significant interactions between treatments involving Lysol, in some of which Lysol appeared to increase the severity of lesions, and in others to decrease their severity (e.g., Table 1, row 1, column 2). One instance in Table 1 indicates a direct reduction in yield due to dipping in Lysol (row 4, column 7, compare yields at 85°F.).

Possibly in these cases Lysol killed organisms antagonistic to the main pathogen, *Fusarium*. The principal antagonists for *Fusarium* noticed in plate cultures from corm lesions have been bacteria. Also bacteria have occasionally been found in hand sections taken from the inner margins of static Fusarium lesions. Lysol is known for its bactericidal action, which possibly explains this anomalous result of dipping.

Interaction of curing and injury from a mercurial dip.—A form of interaction which explains other erratic results of dipping gladiolus corms became evident in experiments with a mercurial fungicide, New Improved Ceresan (ethyl mercury phosphate). Treated corms were dug and examined for signs of chemical injury after roots and shoots had emerged. Some vegetative buds had been injured, but more serious was the killing of tissue inward from the basal scar. When the killing extended to the origins of emerging roots or cut across the vascular bundles leading to the root tips, the roots failed to grow, or were thin and weak. Partial destruction of the root system may cause stunting of vegetative growth even when shoots emerge normally.

If before dipping in New Improved Ceresan the corms were effectively suberized by curing at high temperatures, there was little or no chemical injury. If the corms had been cool-stored without previous curing, New Improved Ceresan produced the characteristic killing of tissues inward from the basal scar. No periderm formed inside the zone of dead and injured tissue; and judging by the progressive killing, for a time mercury appeared to be continually absorbed further into the tissue.

In 1 field trial, curing at 95°F. for a week just before planting protected a poorly suberized and badly diseased lot of corms from chemical injury. Few plants of the treated but non-cured checks emerged. The cured and treated corms produced normal and relatively healthy plants. In other field and greenhouse trials emergence counts, measurements of shoot growth, and production of flowers have indicated that New Improved Ceresan can be applied with benefit and without injury to well-cured corms.

In farming practice it has been observed that properly suberized corms may be dipped before planting in New Improved Ceresan without any bad effect on the subsequent crop. Some degree of injury has generally followed treatment with New Improved Ceresan of poorly cured corms, even when the dip was properly used and the corms planted immediately in moist soil. These experiments and observations help to explain the lack of conformity between the effects of fungicidal dips on gladioli from different sources under different climatic conditions (3). Corms harvested in a cool climate and inefficiently cured may suffer chemical damage from mercurial fungicides such as New Improved Ceresan that outweigh their fungicidal effect. Corms harvested in a hot climate, for example in Florida, may be subjected to temperature conditions more favorable for efficient curing. In such regions use of New Improved Ceresan may cause less chemical damage to gladioli and give more efficient protection to the growing plants.

Comparison between Lysol plus ferbam and New Improved Ceresan.—New Improved Ceresan applied to gladiolus corms immediately before planting still appears the most efficient fungicide for the field control of *Fusarium oxysporum* f. *gladioli* (Massey) Sny. and Hans. Lysol plus ferbam has some fungicidal action on *Fusarium*, but when tested in the field 2 seasons in succession on the same lot of corms it was on the average inferior to New Improved Ceresan. New Improved Ceresan has also given better control of the neck rot phase of *Botrytis*, and in 1 trial it gave a significant reduction in Botrytis lesions on the daughter corms.

On the other hand, it was suggested above that Lysol plus ferbam was efficient in controlling secondary invaders of lesions initiated by *Botrytis* and other pathogens, or by injuries. In this it seems to be at least

as effective as New Improved Ceresan. Some evidence from both greenhouse and field trials suggested that Lysol plus ferbam might also be as effective against *Fusarium* as mercurial fungicides when corms were cured under cool conditions and grown during winter, spring, and early summer.

Results of a test made 2 years in succession with the same stock of gladioli gave evidence that Lysol plus ferbam was more effective than New Improved Ceresan in controlling Rhizoctonia disease of gladiolus. In southern California *Rhizoctonia* causes shredding of the leaf bases and corm husks, and carries over to the cormels, causing a serious disease in plants grown from them. Lysol plus ferbam applied each year reduced the *Rhizoctonia* to negligible amounts. Applied to the parent corms of the first planting, it protected the plants for 2 seasons as effectively as New Improved Ceresan applied to the parent corms during both seasons.

The field trials quoted above illustrate the same general principle as the greenhouse trials: that the different fungicidal materials applied to gladiolus corms have specific effects on various organisms or various manifestations of disease.

DISCUSSION.—A combination of pretreatment (curing) and dipping the corms in an efficient fungicide seems necessary as a routine measure for the control of corm-borne diseases of gladiolus. In the present work fungicidal treatments were confined to dipping immediately before planting, but recent work elsewhere (5) and results obtained by southern California growers (1) suggest that a fungicidal treatment may be given immediately after the corms are cleaned and before they are put in cool storage.

The erratic and uncertain effects of fungicide dipping have found an explanation in the number of variables that may change the efficiency of the fungicides incorporated in the dips. The logical way to reduce these variables to unimportance is to buffer the system consisting of corms, invading organisms, and fungicide against the effects. This may be partly accomplished by raising the natural defenses of the corms to a level that will protect them under a wide range of conditions against both unfavorable environments and the further action of destructive organisms: more specifically by proper curing (1). Also, the environment to which the corm is exposed may be controlled in some measure by proper cultural practices at all stages, and by proper storage of the corms. Fitted into such a framework, fungicidal dips may effectively and consistently control corm-borne diseases. Ignorance or neglect of the framework inevitably results in unpredictable results from the use of fungicidal dips.

SUMMARY

A study was made of interactions between dipping gladiolus corms in fungicidal materials, pretreatment at different temperatures, and the conditions under which the corms were subsequently grown. Current effects on the development of disease symptoms during growth were studied, and in part these were correlated with effects on yield.

In the main 2 materials, Lysol and ferbam, singly and in combination, were used in the study of interactions between the effects of dipping, pretreatment, and the conditions of sprouting.

The action of Lysol on the expansion of lesions and finally on yield was greatly influenced by the state of the corms when they were dipped, i.e., by previous curing conditions, and by the conditions under which the corms were subsequently sprouted. Lysol sometimes decreased disease symptoms and increased yield, but at times it tended to increase symptoms and decrease yield. Suppression by Lysol of secondary invaders or of possible antagonists of the most active pathogen, *Fusarium*, was suggested to explain these opposite effects.

Ferbam apparently acted directly on *Fusarium*, and had little significant effect on secondary invaders and antagonists in disease tissues.

Each kind of fungicide had an effect on the expansion of some types of lesion but not of others. These effects were reflected in final yield.

Injury to treated corms due to toxicity of a mercurial dip was prevented by previous curing and the development of periderm over exposed surfaces. Injury is due to the absorbtion of mercury through poorly suberized surfaces.

Comparison of New Improved Ceresan with Lysol plus ferbam showed each dip to have specific effects on different pathogens and different types of corm lesions.

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THE DIFFERENTIATION OF FIREBLIGHT RESISTANT SEEDLINGS WITHIN PROGENIES OF INTERSPECIFIC CROSSES OF PEAR [1]

T. R. Carpenter and J. R. Shay [2]

This paper presents 1) a new method of inoculating pear seedlings with *Erwinia amylovora* (Burr.) Winslow et al., 2) studies of the optimum age and temperature for inoculating resistant pear seedlings to obtain maximum infection and 3) the effect of hardness of host tissue on disease development. The method commonly used to distinguish susceptible plants in segregating populations is to inoculate with a hypodermic syringe during the first 3 seasons of growth in the nursery. This method is laborious and undoubtedly retards fruiting of the trees due to adverse effects of crowding and delayed transplanting. The technique employed in differentiating apple seedlings susceptible to *Venturia inaequalis* (Cke) Wint. by greenhouse inoculations within a few weeks after the seedlings emerge (2, 13) suggested inoculation trials at a similar growth stage for eliminating fireblight susceptible seedlings in the pear breeding program.

MATERIALS AND METHODS.—Two cultures of *E. amylovora* were used interchangeably for all inoculations reported herein. Both cultures were isolated by Dr. H. W. Anderson, University of Illinois, Urbana, Illinois in the summer of 1947; number 109, was from an artificial infection of an open-pollinated seedling of Pai Li, (*Pyrus ussuriensis* Maxim.) and number 111 was isolated from a natural infection of *P. serotina*, Rehd. Cultures of the organism were maintained and increased on nutrient dextrose or sucrose agar. Inoculation of immature pear fruits with a pure culture of the organism under aseptic conditions proved to be another satisfactory method for perpetuation of the organism. Cultures on slants of solid medium covered with sterile mineral oil and held at 6 to 8°C. retained pathogenicity for periods up to 18 months. The pathogenicity of all cultures was tested on open-pollinated Bartlett seedlings before being used as inoculum. Inoculum was prepared by suspending bacteria from 24 hour cultures in distilled water.

Highly satisfactory inoculum was prepared directly from infected host parts as follows: Recently infected plant parts were removed, surface sterilized in 1:1000 $HgCl_2$, cut into $\frac{1}{8}$ to $\frac{1}{2}$-inch sections, placed in sterile water, and subjected to reduced pressure by use of a water-type filter pump for approximately 20 minutes to increase outward diffusion of the bacteria. The liquid portion was then filtered through a double-thickness of cheesecloth.

The host plants used in this investigation[3] were seedlings from controlled and uncontrolled pollinations of seedling selections and named varieties of *Pyrus ussuriensis* Maxim., *P. serotina* Rehd., and *P. communis* L.

The hybrid seeds were removed from the fruit in September of the year of the cross, afterripened for 12–15 weeks at 36°–42°F. then planted in new vermiculite in greenhouse flats. Upon emergence, the seedlings were transplanted to soil in 3-in. pots.

The plants were transplanted to nursery rows as soon as weather permitted in the spring. An overhead sprinkling system was available for irrigation when needed. All nursery plots were fertilized heavily with NH_4NO_3 soon after growth had started in spring. First inoculations in the nurseries were made soon after vigorous growth had started, usually during the first week of June. Escapes and resistant individuals were reinoculated at 2-week intervals throughout the growing season.

Hypodermic needles from 27 to 22 gauge and 5 cc. Luer syringes were used in all inoculations in the greenhouse and in the field in which the testing of host resistance was the principal objective. The needle insertion did not interfere with growth of the seedlings since the injection of distilled water resulted in only a tan colored scar on the epidermis of the stem after 3 days. Inoculum was injected into succulent tips $\frac{1}{2}$-in. or less below the growing point.

Spray techniques for introducing the organism into host tissue were investigated in 1950. Water suspensions of bacteria diffused from recently infected host tissue were used as inoculum. Sprinkling inoculum on young seedlings failed to infect the host in each of 5 tests. Inoculum applied at 15 lb. air pressure to terminal leaves and succulent stem tips by use of a DeVilbiss atomizer resulted in 10 per cent of plants infected. The addition of carborundum, 600 grit, at the rate of 10 gm. per liter of inoculum resulted in 20 per cent of plants infected. In August, 1950, the equipment[4] shown in Figure 1 attached to a cylinder of nitrogen gas was used to inoculate 2-year-old trees in the nursery. These trees did not have succulent stem tips. Inoculum without carborundum sprayed at 50 lb. pressure on terminal leaves and stem tips resulted in 29 per cent of trees infected, and the addi-

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[3] The cooperation of L. F. Hough and C. J. Birkeland of the New Jersey and Illinois Agricultural Experiment Stations, respectively, in providing host material for these studies is gratefully acknowledged.

[4] Dr. A. L. Anderson, U.S.D.A., Div. Fruit and Vegetable crops, Michigan State College, East Lansing, Michigan, suggested this equipment.

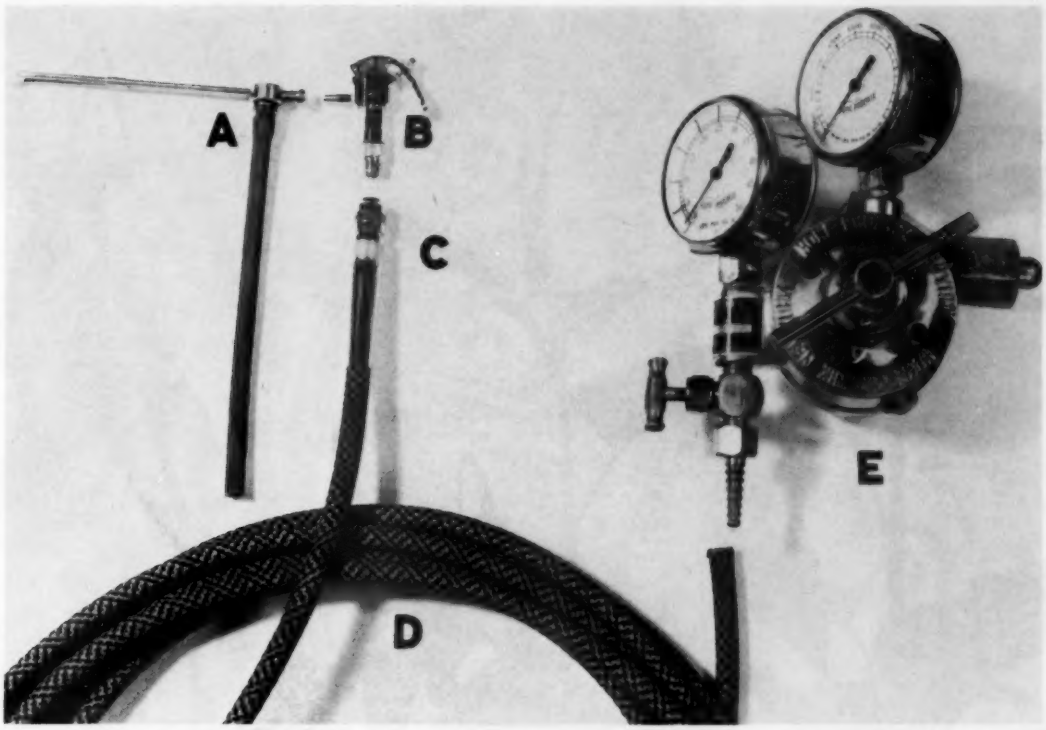

FIG. 1. Equipment for spray-type inoculation. A) De Vilbiss No. 16 atomizer. B) De Vilbiss compressed air cut-off No. 631. C) De Vilbiss cut-off connection No. 634. D) High pressure hose. E) Hoke-Phoenix 2-stage reducing valve No. 901.

tion of carborundum to the inoculum resulted in 39 per cent of the trees infected. A comparable lot of trees inoculated with a hypodermic needle resulted in 40 per cent infection.

Symptoms in the greenhouse and in the field appeared 1–9 days following inoculation. Progress of the organism was apparently arrested within 30 days in the majority of resistant seedlings. However, the progress of the organism was extremely slow and erratic within many individuals. The following infection classes were established, and each inoculated plant was rated on the basis of the most severely infected shoot or stem at the end of the greenhouse or field season:

0. *Immune.* No evidence of disease development. (Fig. 2, A)

1. *Highly resistant.* The organism progressed not more than 2 leaves from point of inoculation in either young seedlings or older plants.

2. *Moderately resistant.* Progress of organism arrested below the second leaf from point of inoculation and above the first true leaf in young seedlings (Fig. 2, B) or, in older plants, necrosis involving not more than half the length of the previous year's growth.

3. *Slightly resistant.* Progress of organism arrested just short of the cotyledonary node in young seedlings or just above the ground line in nursery plants; survival of the plant doubtful.

4. *Susceptible.* Necrosis of stem extended below cotyledonary node in young seedlings or the ground line in older plants; plants dead or dying (Fig. 2, C). For comparing treatments, disease indexes were computed by averaging the infection class values (0 through 4) for the seedlings in a given treatment.

GROWTH STAGE AT WHICH RESISTANCE IS EXPRESSED BY YOUNG SEEDLINGS.—At the outset of the investigations, it was necessary to determine the earliest stage in growth of the seedlings in the greenhouse at which resistance to the organism is manifest. In the greenhouse, 1948 and 1949, seedlings were inoculated at different stages of growth. The data are assembled in Table 1.

The seedlings were killed in all but 1 test when inoculated before the 8-leaf stage. Several immune seedlings could be selected beginning with the 8-12-leaf stage. Intermediate resistance was not consistently demonstrated in the seedlings until the 18-24-leaf stage. At 1 year of age, the seedlings displayed much greater resistance than at any earlier stage. This gain in resistance with age was quite marked.

Practically all the immune seedlings selected from inoculations made between the 8-18-leaf stage continued to exhibit immunity in subsequent nursery inoculations. Seedlings of *P. ussuriensis*, 76, which exhibited immunity at the 18-24-leaf stage, were remarkably consistent in their disease rating following inocu-

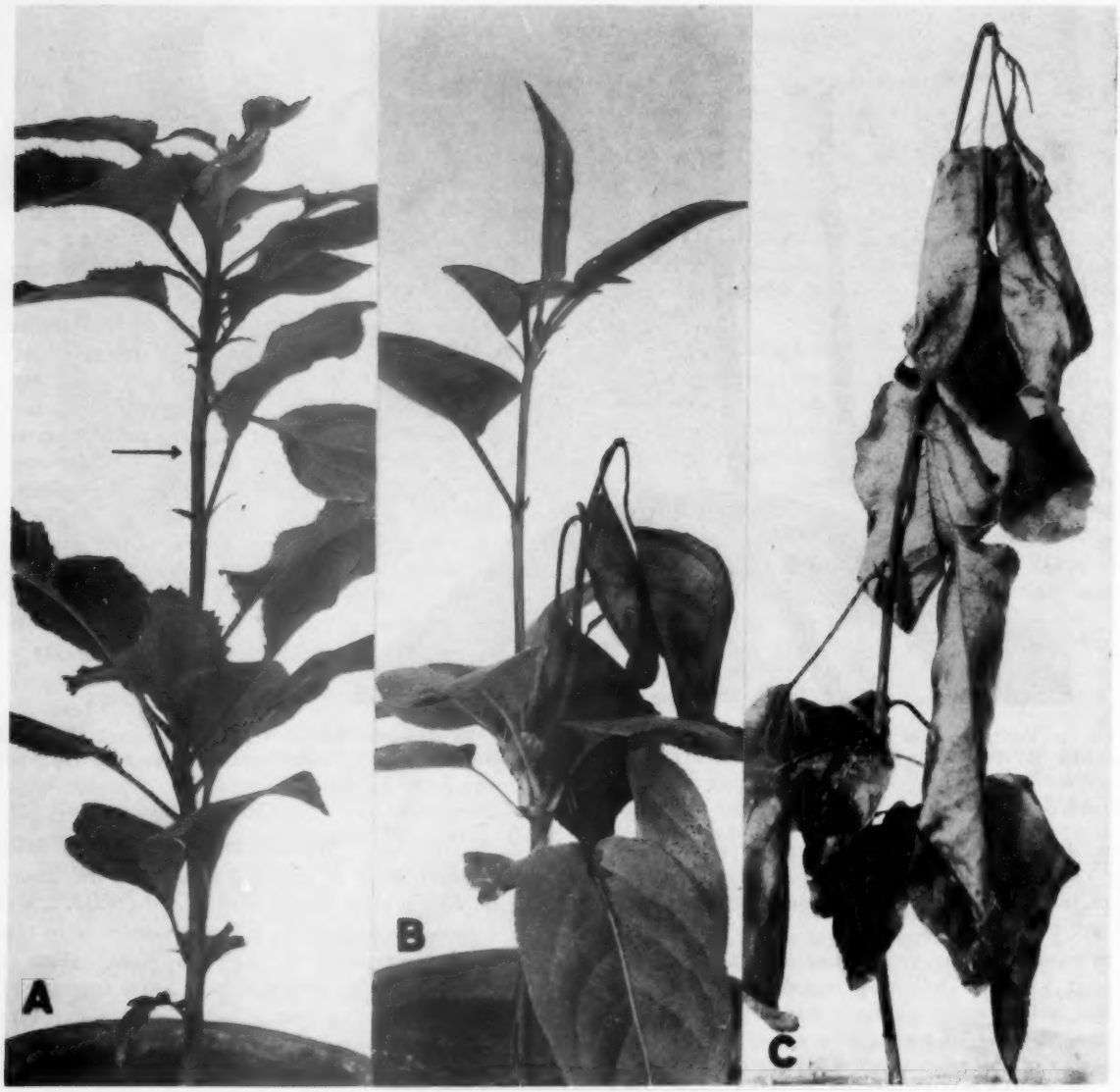

FIG. 2. Infection classes demonstrated by pear seedlings following inoculation with *E. amylovora*. A) Immune (class 0) demonstrated by *P. ussuriensis*, 76 o.p. seedlings. Arrow indicates point of inoculation. B) Moderately resistant (class 2) demonstrated by a Pai Li o.p. seedling. C) Susceptible (class 4) demonstrated by Pai Li o.p. seedling.

lations in the nursery. Similar seedlings of Pai Li were more variable in their reaction to nursery inoculations. In both parentages, seedlings which exhibited intermediate resistance at the 18-24-leaf stage yielded several susceptibles in later nursery inoculations but there was a significant trend toward greater resistance with age.

GREENHOUSE SEEDLINGS VS. FIRST YEAR NURSERY INOCULATIONS.—In 1948, the seedlings of 3 progenies (Pai Li, o.p.; *P. ussuriensis* 76 × Favorita; and *P. ussuriensis* 76, o.p.) were divided into 2 groups. One group of seedlings received the initial fireblight inoculation at an early growth stage in the greenhouse, and the other group at a later growth stage after transplanting to the nursery. Each group received the routine inoculations in the nursery during the 1949 and 1950 growing seasons. The disease indexes of the 2 groups for each of the 3 seasons of testing are presented in Table 2.

The disease indexes show highly significant differences between the 2 treatments following the initial inoculation in 1948. A greater degree of susceptibility was displayed by the early (greenhouse) inoculated group. In 1949 and 1950, after 1 and 2 years respectively, of growth in the nursery, the differences between the 2 lots of plants were nonsignificant in each of the 3 progenies.

In 1950, prior to the first inoculation in the nursery, only 6 trees of the 1948 early (greenhouse) inoculated group had active holdover cankers. These 6 trees died

TABLE 1.—*The effect of age of pear seedlings at time of initial inoculation with E. amylovora on disease development*

Progeny no. and cross	Age initially inoculated[a]	Seedlings tested	Seedlings in stated infection classes: immune (0)	resistant (1,2,3)	susceptible (4)	Disease index
		No.	No.	No.	No.	
1948 test:						
1. Pai Li o.p.	2-8	73	0	0	73	4.0
	8-12	100	1	4	95	3.9
	18-24	104	4	64	36	2.6
	1 yr.	177	62	109	6	1.4
2. *P. ussuriensis*, 76 × Favorita	2-8	53	0	0	53	4.0
	8-12	104	3	0	101	3.9
	18-24	104	2	81	21	2.4
	1 yr.	137	84	51	2	0.8
3. *P. ussuriensis*, 76 o.p.	4-6	32	0	0	32	4.0
	8-12	17	6	0	11	2.6
	12-16	83	3	0	80	3.9
	18-24	91	8	68	15	2.2
	1 yr.	26	19	7	0	0.5
4. *P. ussuriensis*, 76 × P.I. 55805	4-8	62	0	0	62	4.0
	12-16	91	1	0	90	4.0
	18-24	84	1	59	24	2.6
5. *P. ussuriensis*, 98 o.p.	2-8	69	0	0	69	4.0
	1 yr.	39	32	2	5	0.6
1949 test:						
16. *P. ussuriensis*, 76× Bartlett	6-8	78	0	65	13	2.4
	18-26	172	6	162	4	2.1
13. Pai Li × Anjou	18-26	300	—	—	—	2.1
	2 yr.	51	—	—	—	1.9
14. Pai Li × Duchess	18-26	113	—	—	—	2.0
	2 yr.	25	—	—	—	1.6
12. Pai Li × Lincoln	18-26	253	—	—	—	1.6
	2 yr.	58	—	—	—	0.8
24. *P. ussuriensis*, Harbin o.p.	18-26	171	—	—	—	2.1
	2 yr.	108	—	—	—	0.7

[a] Age given as leaf stage (greenhouse) unless designated by years.

during the season. However, 26 trees of the 1948 later (nursery) inoculated group had active holdover cankers in 1950, and 18 of these trees died during 1950. It is concluded that the early (greenhouse) inoculations removed more susceptible individuals than initial inoculations later in the nursery. It would appear that pear seedlings which show high resistance after receiving initial inoculations at early leaf stages in the greenhouse, followed by repeated inoculations in the nursery during the summer, could be selected for trans-

TABLE 2.—*The effect of early (greenhouse) vs. later (nursery) initial fireblight inoculations on disease development in subsequent seasons*

Progeny number, cross and initial inoculation groups	Seedlings tested	Disease indexes at close of indicated season: 1948[a]	1949[b]	1950[b]
	No.			
1. Pai Li o.p.				
Early (greenhouse)	104	2.7	1.7	1.1
Later (nursery)	177	1.4	2.0	1.1
2. *P. ussuriensis*, 76 × Favorita				
Early (greenhouse)	104	2.5	2.0	0.8
Later (nursery)	137	0.8	2.2	0.9
3. *P. ussuriensis*, 76 o.p.				
Early (greenhouse)	91	2.2	1.1	0.5
Later (nursery)	26	0.5	1.5	0.6

[a] P is less than 0.01 (significant) for the difference between the 2 indexes within each of the 3 progenies.
[b] P is greater than 0.10 (nonsignificant) for the difference between the 2 indexes within each of the 3 progenies.

TABLE 3.—*The effect of cool and warm minimum greenhouse temperatures on disease ratings of open-pollinated Ming seedlings*

Minimum temperature	Inoculation at 18-24 leaf stage in greenhouse, 1949		Inoculation during second year in nursery, 1950	
	Seedlings tested	Disease index[a]	Two-year-old trees tested	Disease index[b]
	No.		No.	
60	155	1.5	147	0.8
80	94	2.1	73	0.7

[a] P is less than 0.01 for the difference between these 2 indexes.

[b] P is approximately 0.57 (nonsignificant) for the difference between these 2 indexes.

planting in the test orchard at 1-year of age.

TEMPERATURE EFFECTS ON GROWTH AND DISEASE RATING.—In 1949, 2 comparable groups of Ming o.p. seedlings were grown, inoculated and incubated in the greenhouse at 2 minimum temperature levels of 60° and 80°F. These temperatures were maintained except for short periods during bright days. Data in Table 3 show a significantly higher disease rating for the plants held at 80°F. in 1949. Inoculations of these same seedlings the following season in the nursery showed no significant difference between the 2 temperature treatments.

In 1950, seedlings of 6 progenies, 31 Bartlett o.p., 30 Kieffer o.p., 27 Ming × Duchess, 26 Ming × Bosc, 25 Ming × Bartlett, and 29 Ming × Meigetsu, were divided into 4 groups and were grown, inoculated and incubated in temperature compartments at the following temperatures: 70°, 78°, 84°, and 91.5°F. The seedlings were placed in the compartments at the time of transplanting from vermiculite to soil. Each compartment (a 3-ft. cube) accommodated 100 3-inch pots and was constructed of Wire-O-Glass stretched on wooden frames and placed in a greenhouse held at 65-70°F. The desired temperature, ± 1.5°F. (except for brief periods on bright days) was maintained by 2 250-watt, flat space heaters controlled by a Fenwal Cartridge-type, tension-operated thermoswitch. In each compartment daylight was supplemented by 4 60-watt Lumiline light tubes to provide a 16 hour day. Most of the plants grew best at 70°F. and 78°F. The Ming crosses, except Ming × Duchess, grew better at 78°F. than at 70°F. (Table 4).

From the disease indexes (Table 4) the 91.5°F. temperature was less favorable for all progenies than one of the lower temperatures. Between the 70 to 84°F. range there was no one temperature that was consistently best for disease development in all progenies.

EFFECT OF HARDNESS OF HOST TISSUE ON DISEASE EXPRESSION.—The lack of succulent stem tissue was suspected of being a major factor in determining the expression of resistance after certain initially immune reactors proved susceptible in subsequent inoculations. In the 1948 greenhouse tests, 93 seedlings of Pai Li parentage were inoculated with proven pathogenic inoculum after the plants had ceased growth temporarily and hardened. No infection resulted. Later in the season after terminal elongation had resumed and the

TABLE 4.—*The effect of temperature on growth and susceptibility to fireblight of pear seedlings in the greenhouse, 1950*

Progeny No. and Cross	Temperature (°F.)	Number seedlings tested for growth	Average growth (inches)	Number seedlings tested for disease development[a]	Disease index
31. Bartlett o.p.	70	24	2.4	17	3.2
	78	24	2.2	16	3.5
	84	24	1.4	16	3.4
	91.5	21	1.5	15	2.3
30. Keiffer o.p.	70	25	2.9	19	2.2
	78	23	2.9	14	2.4
	84	24	2.2	17	2.1
	91.5	22	1.6	17	1.8
27. Ming × Duchess	70	24	1.6	16	2.4
	78	24	1.6	16	3.1
	84	25	1.3	20	3.0
	91.5	25	1.0	17	2.6
26. Ming × Bosc	70	10	1.7	5	2.8
	78	10	2.4	5	2.0
	84	8	1.2	5	2.2
	91.5	10	1.3	4	1.8
25. Ming × Bartlett	70	15	2.3	12	3.2
	78	15	2.7	13	3.2
	84	15	1.5	11	2.5
	91.5	13	1.1	8	2.8
29. Ming × Meigetsu	70	15	2.6	10	3.4
	78	14	2.8	10	3.2
	84	10	1.8	7	1.9
	91.5	14	1.5	9	2.3

[a] Inoculated with *E. amylovora* at the 18-leaf stage.

TABLE 5.—*The effect of cessation of terminal shoot growth in resistant pear seedlings of fireblight development following artificial inoculation*

Progeny No. cross and date of first 1950 inoculation	Number 2-year-old seedlings tested[a]	Disease index
12 Pai Li × Lincoln		
July 3[b]	25	1.6
July 13[b]	114	1.1
13 Pai Li × Anjou		
July 3	20	2.1
July 13	216	1.7
14 Pai Li Duchess		
July 3	20	2.2
July 13	77	1.8
15 Pai Li × Bartlett		
July 3	22	2.2
July 13	146	1.6
16 *P. ussuriensis*, 76 × Bartlett		
July 3	18	1.5
July 13	114	1.0

[a] These 2-year-old seedlings were in infection class 2 the previous year.

[b] Terminal shoots were growing vigorously on July 3 but had ceased growth on July 13.

plants were reinoculated, 65 of these plants became infected. In October, 1950, 216 seedlings of Ming parentage, 137 Kieffer o.p. seedlings and 59 Bartlett o.p. seedlings were inoculated while they were in the 16–18 leaf stage in the greenhouse. These plants had ceased terminal elogation 3 weeks prior to inoculation following a period of several days in which the temperature was above 90°F. The only plants to become infected were 4 of the Ming crosses, 2 of the Bartlett o.p., and 1 of the Kieffer o.p.

In 1950, 105 2-year-old trees which had reacted as moderately resistant (infection class 2) in 1949 were inoculated July 3 while the current season's growth was succulent. On July 13, 10 days later, 667 similar trees were inoculated after mature tissue had developed in the current season's growth. The results are in Table 5. From the disease indexes (Table 5), it is apparent that the earlier inoculation in succulent stem tissue provided a more satisfactory test.

DISCUSSION.—The fireblight resistance present in the oriental pear, *P. ussuriensis*, has been considered to be the highest type present in the genus (4, 5, 11). It is unfortunate that seedlings of varieties such as Kieffer, with somewhat lower levels of resistance, were not available for the first 2 years of this study. Previous workers, so far as can be found in the literature, used seedlings not less than 1 year old (3, 11). Results of this study indicate that the 8-18-leaf stage is critical in *P. ussuriensis* seedlings for the development of resistance to fireblight. Nearly 100 per cent of the seedlings were killed when inoculated prior to the 8-leaf stage. Immune seedlings could be detected between the 8– and 18–leaf stages, but seedlings with intermediate types of resistance (infection classes 2 and 3) did not consistently appear until the 18–24 leaf stage. Inoculation at the 18–24–leaf stage eliminated a higher percentage of susceptible (infection class 4) seedlings than inoculations at the 1-year-old stage in the nursery.

In spite of early greenhouse inoculations, some seedlings escaped infection. A more vigorous, succulent type of growth was obtained, and a higher percentage of susceptible seedlings was eliminated in most progenies at 70°–84°F. than at 91.5°F. This agrees with results obtained by Miller (6) and Shaw (12). The demonstrations that succulent terminal tissue is a factor of major importance in evaluating the true resistance of pear seedlings to fireblight in an inoculation program is in full agreement with other workers (1, 3, 6, 7, 8, 9, 10, 11, 12, 14).

It would seem advisable to grow resistant greenhouse segregates for a full year in the nursery with inoculations during the growing season before selections are made for transplanting in the test orchard. This would aid in the elimination of seedlings which permit development of holdover cankers and of those susceptible to winter injury. Final judgment on the efficiency of the greenhouse test for removing susceptible seedlings must await a test of floral infection.

SUMMARY

Seedlings of controlled and uncontrolled crosses of *Pyrus ussuriensis* Maxim and *P. serotina* Rehd. parentages were used to determine the success of separating fireblight-resistant from susceptible seedlings by inoculations with *Erwinia amylovora* (Burr.) Winslow et al. in early seedling stages in the greenhouse. The relative efficiency of this method was compared with the laborious nursery inoculation method generally used.

No appreciable resistance was expressed prior to the 8-leaf stage.

Individuals having intermediate types of resistance could be detected at the 18–24–leaf stage. The general resistance level of all populations increased with age of the seedlings.

A higher percentage of susceptible plants was eliminated in early (greenhouse) growth stages than was eliminated from similar progenies initially inoculated at later growth stages in the nursery.

At 3 years of age, there were many more holdover cankers among the trees initially inoculated at later growth stages in the nursery than among the trees initially inoculated at early growth stages in the greenhouse.

As found by previous workers, the fireblight bacteria must be placed in succulent tissue for optimum infection. Woody mature stem tips of susceptible seedlings may be resistant to infection.

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FURTHER STUDIES ON BARLEY POWDERY MILDEW AS INFLUENCED BY ENVIRONMENT [1]

V. F. Tapke [2]

Apparently unlike any other plant disease, powdery mildew of wheat, oats, and barley (*Erysiphe graminis* D.C.) has been found severe in hot, cold, wet, and dry seasons (19), a situation difficult to understand. Recently the writer (19) reported that susceptibility to mildew in wheat and barley is stepped up when plants are grown under conditions that favor a lush, tender growth such as moderately cool, moist air, ample watering, moderate intensity of light, and liberal nitrogen fertilization. On the contrary, exposure to severe cold, heat, drought, long intense light, and other conditions that toughen the tissue promotes resistance. Outbreaks of powdery mildew, therefore, seem tied in with the tenderness or toughness of the plant tissue. Combinations of many environmental factors over the growing period determine the type of tissue that the plant develops. This seems to explain why no fixed relationship exists between epidemics of powdery mildew and short periods of a particular kind of weather.

In the further study with barley presented here, environmental conditioning played an important role also in the expression of adult resistance to powdery mildew and in the infection gradient within a plant.

Adult resistance.—Changes in the resistance or susceptibility to disease accompanying advances in age have been reported in various crops (10, 13, 16, 22). Varieties of wheat and barley that are highly susceptible to mildew in the seedling stage may become resistant in the adult stage of growth (7, 12, 20). Apparently the change generally has been regarded as a genetic phenomenon associated with the ontogenetic development of the plant. Although such varieties might be expected to have less value as breeding material than varieties known to be resistant or immune throughout their entire life, the development of adult resistance may have considerable practical value. As noted by Smith and Blair (18), it may be effective in standard varieties when mildew intensity is at the maximum and it may be relatively simple to transfer to more susceptible varieties.

Graf-Marin (7) in 1934 and Tidd (20) in 1937 presented enlightening reports on adult resistance to powdery mildew in barley. Their data indicated, however, that some unknown factor apparently was functioning. Graf-Marin sowed Alpha seed in pots in a greenhouse at Ithaca, N. Y. on February 9, 1930 and inoculated plants March 1, March 15, April 5, April 21, and May 13. On the last date, seedlings of Alpha also were inoculated. The infection of plants thus inoculated 20, 34, 55, 71, and 93 days after seeding, respectively, was heavy, heavy, mild, weak, and none. The seedlings inoculated on May 13 became heavily infected, showing that the environmental conditions then were favorable for mildew. From these and other results Graf-Marin concluded that "the older the barley plants are at the time of inoculation with mildew, the more resistant to infection they are."

The writer has frequently seen fields and plots of adult Alpha barley heavily covered with powdery mildew in the state of New York. Moreover, Alpha plants in the heading stage were inoculated in December 1946 in a greenhouse at Beltsville, Md., and severe infection was obtained. Also at Beltsville, Md., the writer twice repeated the experiment of Graf-Marin under widely different greenhouse conditions, using the same pure line strain of Alpha barley from the Department of Plant Breeding at Cornell. In the first test the plants were grown in pots from October 1947 to January 1948, under preinoculation conditions pro-

[1] Accepted for publication December 14, 1952.

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moting a lush, tender growth, i.e. cool, moist soil and air, short days and low intensity of natural daylight. Greenhouse temperatures were approximately 60°–65° F. during the day and 60° at night. From mid-November to the end of the experiment the natural daylight was supplemented with electric light from 4:30 P.M. to 11:00 P.M. daily. The 100-watt Mazda bulbs, with reflectors, were hung 1 or 2 ft. above the plants and about 5 ft. apart. As in the original test, mildew-free plants were inoculated 20, 34, 55, 71, and 93 days after the seed was sown and seedlings, 1½–2 in. high also were inoculated on the last date. The results were strikingly different from those of Graf-Marin. All leaves of all plants, regardless of age or date of inoculation, became heavily mildewed within 10 days after inoculation. (Fig. 1) When the test was repeated from February to May 1948, however, with dates of seeding and inoculation exactly the same as in Graf-Marins original experiment of 1930, the results were identical with those of Graf-Marin. The infections were heavy, heavy, mild, weak and none, respectively, on the plants inoculated March 1, 15, April 5, 21, and May 13, (i.e., 20, 34, 55, 71, and 93 days after sowing). Young seedlings, 1½–2 in. high, inoculated May 13 became heavily mildewed.

The contrasting results of the October to January and February to May tests were caused by differences in environment that conditioned the plants to reaction to powdery mildew (19). The cool, moist soil and air and the short, subdued daylight in the greenhouse from October to January made the plants very tender, hence, very susceptible. Small grain plants growing in greenhouses during late autumn and early winter usually are so weak and tender they must be supported with stakes. Plants of the same crops grown in the greenhouse in the spring and subjected to warmer, drier conditions and more light develop tougher tissues that are resistant to mildew.

Tidd in 1937 (30) reported experiments that revealed, apparently for the first time, the influence of environment on expression of adult resistance in barley, although he made no reference to environmental plant conditioning. He grew 83 varieties of spring barley in a greenhouse at Ann Arbor, Michigan, from November 1933 to March 1934 and inoculated the plants in March in the late shooting and heading stages. Seedlings of each variety also were inoculated in March for comparison. The reaction of the seedlings and adults of each individual variety was similar. When the test was repeated with 48 of the varieties grown in April and May 1934, the reaction of the seedlings was practically the same as in the previous experiment but the adult plants developed appreciably less mildew than did those grown in winter. Confirmatory results in a further study led Tidd to the conclusion that "given certain environmental conditions, adult barley plants are as susceptible to mildew as are seedling plants of the same varieties."

Further evidence of the influence of seeding date and post-seeding conditions is presented in Table 1. Seed

FIG. 1. Adult plant of Alpha barley heavily infected with powdery mildew, inoculated 93 days after seeding.

of 4 varieties of spring barley was sown in greenhouse pots on the ninth or tenth day of each month from October 1947 through February 1948. Plants from each seeding were inoculated uniformly about 2½ months after seeding when most of the plants were in the heading stage. Seedlings 1½–2 in. high were inoculated at the same time. Mildew readings were taken 2 weeks later. All seedlings became heavily mildewed. In the adult plants (Table 1) infection was severe following inoculation in December but decreased consistently with each later inoculation. The results are similar to those of Graf-Marin (7) and Tidd (20), but

TABLE 1.—*Effects of seeding date on mildew severity in 4 varieties of barley grown in a greenhouse at Beltsville, Md., and inoculated in the heading stage*

Date of Seeding	Date of inoculation	Mildew severity ratings on successive seedings [a] Variety and C. I. No. [b] Alpha C.I. 959	Baku C.I. 709	Oderbrucker C. I. 4666	White Smyrna C.I. 910
October 10, 1947	December 22, 1947	4	4	4	4
November 10, 1947	January 26, 1948	3–4	3+–4	3+–4	3+–4
December 10, 1947	February 25, 1948	1–2	3–4	3–4	3
January 9, 1948	March 24, 1948	1	3	3	0
February 10, 1948	April 22, 1948	0	2	2	0

[a] Ratings are: 0 = highly resistant, 1= very resistant, 2 = moderately resistant, 3 = moderately susceptible, and 4 = susceptible.

[b] C. I. refers to accession number of the Division of Cereal Crops and diseases.

they can be explained by differences in preinoculation environment.

Table 1 also shows differences in varietal expression in the 3 later seedings in which Alphat and White Smyrna developed more resistance than did Baku or Oderbrucker. The reasons for the differences have not been determined.

In the experiments of Graf-Marin (7) Tidd (20), and the writer, the infection of inoculated seedlings was used as the check against adult plant resistance under different conditions. However, Newton and Cherewick (12) reported that mildew development on wheat and barley seedlings kept at constant temperatures was less at 25° C. than at 15° C. The mildew pustules continued to sporulate at 15° C. until the seedlings were killed whereas, at 25° C., the pustules ceased to sporulate. Therefore, at other than optimum temperature for infection, seedling susceptibility might be open to objection as an accurate gauge of the favorableness of environmental conditions for infection.

High temperatures as a post-inoculation factor was eliminated in one experiment in which plants of the susceptible Alpha (C. I. 959) and Oderbrucker (C. I. 4666) varieties were grown in pots at Beltsville, Md., during June, July, and August 1948. Two weeks after the seed was sown, ½ of the pots were placed in an outdoor bed receiving full sunlight, and were watered lightly to develop toughened tissues. The other pots were put in an outdoor cabinet, controlled at 64°–68° F., where they received light through several large glass windows. Ten pots each of Alpha and Oderbrucker from the outdoor bed and from the control chamber were inoculated uniformly in the late shooting and early heading stages in August, then put in the control chamber. (Mildew inoculum, usually scarce in late summer, was obtained in ample quantity from seedlings grown in a glass-walled chamber at 50° F.) Mildew readings were taken 10 days after inoculation. All leaves of the lush, tender plants grown continuously in the cool chamber became heavily infected. The toughened plants grown outdoors under hot, dry conditions produced only sparse mildew on the lower leaves. The experiment was repeated the following summer with the varieties, Alpha (C. I. 959), Oderbrucker (C. I. 4666), and Trebi (C. I. 936). Results were similar to those of the preceding year. The influence of preadult conditions on adult resistance therefore seems clear.

The relative toughness of plant tissue as a factor favoring adult resistance possibly may be related to cuticle development. Graf-Marin found that the cuticle of leaves from young barley plants ranged from 0.40–1.50 μ in thickness whereas that from leaves of adult plants ranged from 2.5–5.0 μ. An abundant infection of mildew was obtained on wounds following the removal of cuticle from leaves of adult plants kept under conditions that prevented the underlying cells from drying. This suggests that the resistance of adult plants to mildew may be merely mechanical. In a study of the powdery mildews of cereals, apples, and roses, Corner (5) obtained results indicating that resistance is due primarily to toxins in the host cell but that environmental and structural factors also may be operative.

THE MILDEW GRADIENT.—In the experiment reported in Table 1 it was observed that increases in adult resistance were not accompanied by uniformly reduced areas of infection on all leaves. The percentage of the leaf area covered with mildew was greatest on the lowest or first leaf with a progressive decrease to the top or latest leaf (Figure 2.) Previously, Graf-Marin (7) noted the scarcity of mildew on the last leaves produced by Alpha barley, and others (1, 2, 8, 17, and 21) have reported a heavier attack of powdery mildew on the lower than on the upper leaves of small grain plants. The term "mildew gradient" will be used hereafter to describe this expression of infection. Apparently only Grainger (8) has attempted to explain it. He notes that oat powdery mildew in the west of Scotland becomes severe when the atmospheric humidity reaches saturation for appreciable periods. He finds also that humidity is highest near the ground. Presumably, therefore, the lower leaves are exposed to atmospheric saturation for a longer period than are the higher leaves.

In a test for a possible relation between mildew gradient and atmospheric humidity, the writer used individual potted plants of Alpha (C. I. 959), Oderbrucker (C. I. 4666) and other susceptible varieties grown in a greenhouse. The plants were uniformly

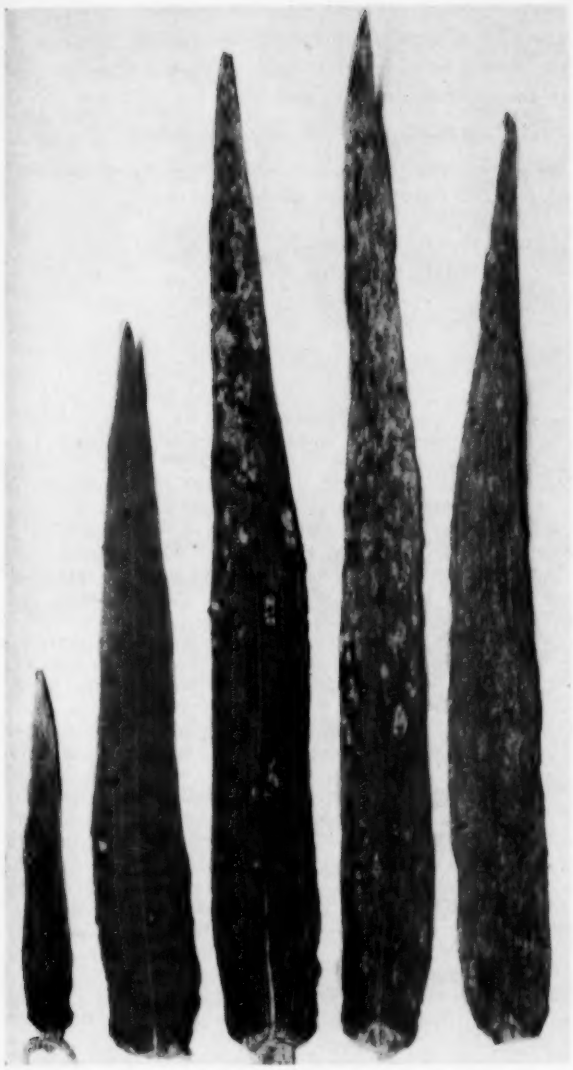

FIG. 2. The 5 leaves of a naturally inoculated, field-grown barley plant showing a gradient infection with powdery mildew. Left to right, top to lowest leaves.

inoculated at heading time in April and May 1948, and single pots were placed on laboratory tables, or in the center of thoroughly dry greenhouse benches or similar locations where the movement of air about each plant was unimpeded. The gradient type of infection developed in every trial. Humidographs placed at the upper and lower levels of individual plants registered atmospheric humidities well below 100 per cent but showed no differences between different levels. This indicates that the mildew fungus can develop in unsaturated atmosphere and that the mildew gradient can occur without an atmospheric gradient in humidity.

In another experiment 10 Alpha (C. I. 959) and 10 Oderbrucker (C. I. 4666) individually potted barley plants in the early heading stage were inoculated in April 1949, and placed in a line on a greenhouse bench. Alternate pots were then inverted and suspended by wires between the normal upright plants. The soil was retained in the inverted pots which were watered daily. The tips of the inverted plants attempted to curl upward after a few days, but were tied to wires. A fairly heavy mildew development on all plants was recorded 3 weeks after inoculation. The normal mildew gradient within each variety, decreasing from the basal to the top leaf, (Figure 2) developed remarkably alike on all upright and inverted plants. Evidently a factor or factors other than gradient in atmospheric humidity is responsible for the gradient type of mildew infection. Physiologic gradients in plants are rather common. Gradients in ash (15) lignin and the methoxyl in lignin (14) have been found in barley. Concentration gradients have been reported in juices of corn (9) privet, chestnut, Philadelphus and Bryophyllum (6). Whether or not some physiologic gradient or a gradient in cuticle thickness is concerned with the mildew gradient apparently is not known.

Heretofore, 5 degrees of host reaction ranging from highly resistant to very susceptible usually have been used to record mildew severity, but 5 different degrees of infection often are evident on 5 leaves of a single plant (Figure 2). A symbol such as M.G. 0–5 meaning mildew gradient, zero to very susceptible, top to bottom leaf, may be necessary for accurate description of infection of this kind.

The writer has observed a strong tendency for adult plants to develop the gradient type of mildew infection. A slight mildew gradient usually is evident even when all leaves are heavily infected. The gradient type of infection invariably has been associated with the development of adult resistance in all of the writer's experiments. No barley plant has been seen in which adult resistance has been expressed as a reduced amount of infection with all leaves carrying an equal load of mildew.

In breeding for mildew resistance the use of varieties as parents having only adult resistance would be hazardous except in an environment that could be depended upon to bring this factor into full expression.

SUMMARY

The development of resistance to powdery mildew (*Erysiphe graminis* D.C.) in the adult stage of susceptible barleys is a variable character that may be greatly affected by the environmental conditions under which the plants are grown in the pre-adult stage.

In the greenhouse at Beltsville, Md., adult plants were as susceptible as the seedlings when inoculated in the early heading stage in December and January. The cool, moist soil and air and short, subdued daylight in the greenhouse from October to January made the plants very tender, hence very susceptible. Adult plants became progressively resistant, however, when inoculated in the heading stage in February, March, April and May. This is attributed to a gradual toughening or decrease in succulence of tissue effected by the longer, brighter, warmer days of the advancing

spring season. Seedlings inoculated at each of the above periods were highly susceptible.

Tender and toughened plants likewise became heavily and lightly infected, respectively, when inoculated in the heading stage at the same time and subjected to a controlled post-inoculation environment highly favorable to mildew.

Varieties differed in degree of adult resistance acquired through environmental conditioning.

In the writer's experiments, adult resistance was expressed as a gradient infection in which the percentage of leaf area covered with mildew was greatest on the lowest or oldest leaf and decreased progressively to the top-most or youngest leaf.

The mildew gradient was not affected by any possible gradient in the relative humidity of the atmosphere at different levels above the soil surface.

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